NovaSeq X Series Integration

The Illumina NovaSeq X Series integration provides preconfigured workflows that map to established lab protocols and steps used with NovaSeq X Series sequencing instruments. The integration also supports the associated library prep kits, reagent kits, and assays used in these protocols.

NovaSeq X Series Integration v1.2 Configuration and User Interaction

The Illumina NovaSeq X Series Integration Package v1.2 supports the integration of Clarity LIMS to Illumina NovaSeq X series instruments.

For instructions on validating and troubleshooting the Illumina NovaSeq X Series Integration, refer to *NovaSeq X Series Integration v1.2 Validation and Troubleshooting* on page 32.

> The configuration provided in this integration has been established to support NovaSeq X Series lab processes. Any configuration changes to protocols or workflows (including renaming protocols, steps, and fields) could break the process.

Overview

The integration includes the following features:

- Preconfigured NovaSeq X Series Sequencing v1.1 workflow that maps to lab protocols and instrument runs.
- The following steps in the NovaSeq X Series Sequencing v1.1 preconfigured protocol:
  - Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.1)
  - Make Bulk Pool (NovaSeq X Series Sequencing v1.1)
  - Dilute and Denature (NovaSeq X Series Sequencing v1.1)
  - Load to Library Tube Strip (NovaSeq X Series Sequencing v1.1)
  - AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.1)
  - AUTOMATED - Analysis Run (NovaSeq X Series Sequencing v1.1)
- Automatic validation of run setup information before sample sheet generation or planned run creation.
- Automated generation of the sample sheet. This sample sheet is used to start the sequencing run on the NovaSeq X Series Control Software through Local run mode.
- Automated creation of a planned run on Illumina Connected Analytics (ICA). The NovaSeq X Series Control Software retrieves the planned run for use with the Cloud run mode.

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- Automated tracking of the NovaSeq X Series sequencing run and parsing of run statistics from BaseSpace Sequence Hub to Clarity LIMS.
- Automated tracking of the NovaSeq X Series analysis run and high level analysis summary from BaseSpace Sequence Hub to Clarity LIMS.
- Support for configuration of multiple secondary analyses with multiple library prep kits (LPKs) and index adapter kits (IAKs) for the planned run. The analysis is either performed by ICA in Cloud mode or by DRAGEN on-board the instrument in Local mode.
- [Optional] Preconfigured Library Prep Validation v2.3.2 workflow used for validation purposes only. The workflow contains a single-step protocol that models the library prep required to produce libraries that are ready for the NovaSeq X Series Sequencing v1.1 workflow. For more information, refer to *NovaSeq X Series Integration v1.2 Validation and Troubleshooting* on page 32.
- [Optional] Clarity LIMS Product Analytics (CLPA) integration that can be used with ICA. The CLPA integration installation and configuration is required. For more information, refer to *Clarity LIMS Product Analytics Integration v1.2 Configuration and User Interaction* on page 2399.

Prerequisites and Assumptions

The NovaSeq X Series Integration v1.2 requires a valid BaseSpace Sequence Hub account with an Enterprise or Professional subscription.

Analysis Configuration Template

The NovaSeq X Series Sequencing v1.1 workflow uses analysis configuration templates (ACTs) to configure secondary analysis for planned runs. Required ACTs must be created using BaseSpace Sequence Hub. The ACT names must be unique. The index adapter kit (the label group in Clarity LIMS) selected in the ACT is created in Clarity LIMS. The same label group must be used in the library preparation step. For more information, refer to *NovaSeq X Series Integration v1.2 Validation and Troubleshooting* on page 32.

Make sure that ACT names created in BaseSpace Sequence Hub do not have leading or trailing spaces. Otherwise, Clarity LIMS can have issues with recognizing the ACT names.

Samples

Samples must go through the library preparation and quantification process before they enter the NovaSeq X Series Sequencing v1.1 workflow. It is assumed that the following steps have completed before samples are assigned to the workflow:

- Samples have been accessioned into Clarity LIMS.
- Samples have been run through QC and library prep.

- Samples have the Molarity (nM) global field set to a value. This value is required for the Calculate Volumes automation in the Make Bulk Pool step.

Sample and library names must use only alphanumeric, dash, or underscore characters.

For more information on sample accessioning, refer to *Sample Accessioning* and *Upload and Modify Samples* in the Getting Started section of the Clarity LIMS Core Functionality documentation.

You can assign samples to workflows automatically, using a routing script, or manually—from the Projects & Samples dashboard. Refer to *Assign and Process Samples* in the Clarity LIMS Core Functionality documentation.

Workflows, Protocols, and Steps

The NovaSeq X Series Integration Package v1.2 includes the following workflows:

- NovaSeq X Series Sequencing v1.1
- Library Prep Validation v2.3.2 (optional, but recommended for validation purposes)

The following table describes the protocols and steps included in these workflows.

Table 1 NovaSeq X Series Integration Package v1.2 Protocols and Steps

Protocol Name	Protocol Purpose	Protocol Steps
	Library Prep Validation v2.3.2 Workflow	
Library Prep Validation v2.3.2	Included for validation purposes only, this protocol models the library prep steps required to advance samples to the NovaSeq X Series Sequencing v1.1 workflow. The label group (index adapter kit) used for library preparation must be the same as the index adapter kit specified in the ACT that is being used. For more information, refer to *NovaSeq X Series Integration v1.2 Validation and Troubleshooting* on page 32. The protocol contains a single step - Library Prep Validation v2.3.2. After this step, a routing script sends the samples to the first step of the NovaSeq X Series Sequencing v1.1 workflow (Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.1)).	1. Library Prep Validation v2.3.2
	NovaSeq X Series Sequencing v1.1 Workflow	

Protocol Name	Protocol Purpose	Protocol Steps
NovaSeq X Series Sequencing v1.1	This protocol models the lab processes of run setup for starting a NovaSeq X Series sequencing and secondary analysis run.	1. Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.1) • The secondary analysis of samples is configured using the ACT. • The labels applied to the samples are validated against the selected ACT so that samples use valid labels. 2. Make Bulk Pool (NovaSeq X Series Sequencing v1.1) • The Calculate Volume script assists with pooling samples and adding resuspension buffers and reagents. • The Validate Analysis Configurations automation validates the ACT of pooled samples to make sure that the secondary analysis configurations remain valid after pooling. 3. Dilute and Denature (NovaSeq

Validation Workflow

The Library Prep Validation v2.3.2 workflow allows for validation of the system after installation is complete. This workflow can be replaced by other custom library preparation workflows. For details, refer to *NovaSeq X Series Integration v1.2 Validation and Troubleshooting* on page 32.

Configure a custom workflow as follows.

1. Select Configuration, then select Lab Work.
2. Add a Routing Script automation to the last Master Step of the library preparation workflow.
3. Select Configuration, then select Automation.
4. Create a new Routing Script automation and update it as follows.
   a. For Channel Name, enter limsserver.
   b. For Command Line, enter the following script:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -u {username} -p {password} -i {stepURI:v2} -l {compoundOutputFileLuid0} script:changeWorkflow \
\
--FIELD_NAME 'Sequencing Instrument' \
--FIELD_VALUE 'NovaSeq X Series' \
--WORKFLOW 'NovaSeq X Series v1.1' \
--STEP 'Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.1)' \
--INPUTS_OR_OUTPUTS 'OUTPUTS'"
```

   c. For Automation Use, select the custom library preparation workflow.

Step Automations

The following table lists the step automations included in the NovaSeq X Series Sequencing v1.1 workflow. Automation details are provided in the documentation for each step.

For details on the validation workflow, refer to *NovaSeq X Series Integration v1.2 Validation and Troubleshooting* on page 32.

Table 2 NovaSeq X Series Sequencing v1.1 Workflow Step Automations

Protocol	Step	Automation
NovaSeq X Series Sequencing v1.1	1. Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.1)	• Register Step Completed[1] • Retrieve ACT Information • Validate Reagent Labels and Apply Selected ACT to Samples and Set Next Step • Validate Sample Names and Retrieve Analysis Configuration Template List and Register Step Started[2]

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Protocol	Step	Automation
	2. Make Bulk Pool (NovaSeq X Series Sequencing v1.1)	• Calculate Volumes • Register Pools and Register Step Completed[1] • Register Step Started[1] • Set Next Step • Validate Analysis Configurations
	3. Dilute and Denature (NovaSeq X Series Sequencing v1.1)	• Calculate Volumes • Register Step Completed[1] • Set Next Step • Validate Inputs Flowcell Type and Register Step Started[2]
	4. Load to Library Tube Strip (NovaSeq X Series Sequencing v1.1)	• Register Step Completed[1] • Set Next Step • Validate Flowcell Inputs and Validate Analysis Configurations and Register Step Started[2] • Validate Library Tube Strip Barcode • Validate Run Setup and Create Planned Run
	5. AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.1)	• Register NovaSeq X RunCompleted and Register Step Completed[1] • Register Step Started and Register NovaSeq X Run and Register NovaSeq X Run Association and Register NovaSeq X RunStarted[1]
	6. AUTOMATED - Analysis Run (NovaSeq X Series Sequencing v1.1)	Not applicable

[1] These automations are required for CLPA support only.
[2] These automations are required for the NovaSeq X Series Sequencing v1.1 workflows and contain additional logic needed for CLPA support. If you would like to remove support for CLPA, contact Illumina Support.

All remaining automations are required for the NovaSeq X Series Sequencing v1.1 workflows.

The Illumina Universal Sample Identifier is a global field that is imported with the Library Prep Validation v2.3.2 and NovaSeq X Series Sequencing v1.1 workflows. Illumina Universal Sample Identifier is a text field that is reserved for CLPA support. The field is optional and not required for this integration.

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Step 1: Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.1)

The Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.1) step uses ACTs to configure secondary analysis for samples. Each ACT contains details related to the secondary analysis (eg, index adapter kit to use, reference genome setting, and so on). You can select an ACT from a list and assign samples to it. A planned run can have multiple ACTs and this step must be repeated for each ACT that is required for the run. For more information on creating ACTs and assigning samples to them, refer to *NovaSeq X Series Integration v1.2 Validation and Troubleshooting* on page 32.

The following automations are configured on this step:

- Validate Sample Names and Retrieve Analysis Configuration Template List and Register Step Started
- Retrieve ACT Information
- Validate Reagent Labels and Apply Selected ACT to Samples and Set Next Step
- Register Step Completed

Validate Sample Names and Retrieve Analysis Configuration Template List and Register Step Started Automation

Automatically triggered on entry to the step, this automation completes the following actions:

- Checks that submitted sample names contain only alphanumeric, dash, and underscore characters.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
script:evaluateDynamicExpression -exp 'submittedSamples.each { sample -> if (
!sample.name.matches( ::[A-Za-z0-9_-]+:: ) ) { fail ( ::Submitted sample :: +
sample.name + :: should only have alphanumeric, dash, and underscore characters.
Please rename and try again.:: ) } }' -log {compoundOutputFileLuid1}
```

- Retrieves the list of ACTs that were created in BaseSpace Sequence Hub.

```
/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/novaseqxserie
s-automation.jar script:retrieve_act_list -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1}
```

- Registers the start time of the step by publishing messages to CLPA through the ICA.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s
'com/illumina/upa/scripts/common/step_started.groovy'
```


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Retrieve ACT Information Automation

Automatically triggered when you select Retrieve ACT Information on the Record Details screen, this automation completes the following actions:

- Retrieves the details of the selected ACT and populates the step custom fields (eg, Analysis Version, Library Prep Kit, and so on).
- Saves the details into a file that is available to download. This file is stored in the Analysis Configuration Metadata file placeholder.

```
/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/novaseqxseries-automation.jar script:retrieve_act_info -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -a '{udf:Analysis Configuration Template}'
```

Validate Reagent Labels and Apply Selected ACT to Samples and Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation completes the following actions:

- Checks that the molarity of all samples is specified.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'if (!input.hasValue (::Molarity (nM)::)) { fail(::The Molarity cannot be empty.::) ; } else if (input.::Molarity (nM):: <= 0) { fail(::The Molarity cannot be negative or zero.::) ; }' -log {compoundOutputFileLuid1}
```

- Checks that all samples are indexed and for invalid indexes. The indexes applied are the same as the selected ACT.

```
/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/novaseqxseries-automation.jar script:validate_indexes -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -a '{udf:Analysis Configuration Template}'
```

- Assigns the selected ACT to all samples in the step.

```
/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/novaseqxseries-automation.jar script:assign_act -i {stepURI:v2} -u {username} -p {password} -a '{udf:Analysis Configuration Template}' -l {compoundOutputFileLuid1}
```

- Sets the next step for samples to ADVANCE, which advances them to the next step in the protocol (Make Bulk Pool (NovaSeq X Series Sequencing v1.1)).

```
nextStep = ::ADVANCE:
```

Register Step Completed Automation

Automatically triggered on exit of the step, this automation registers completion time of the step by publishing messages to CLPA through ICA.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s 'com/illumina/upa/scripts/common/step_completed.groovy'
```

Master Step Fields

The following table lists the configuration details for fields that are defined on the Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.1) step.

Table 3 Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.1) Master Step Field Configuration

Field Name	Field Type	Options
Analysis Configuration Template	Text Dropdown	Required Field
Application	Text	Read Only
Application Version	Text	Read Only
Index Adapter Kit	Text	Read Only
Library Prep Kit	Text	Read Only
Reference Genome	Text	Read Only
Secondary Analysis Mode	Text	Read Only

Global Fields

The following table lists the global custom fields that are configured to display on the Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.1) step.

Table 4 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
Molarity (nM)	Numeric		Decimal places displayed = 2

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Field Name	Field Type	Options	Additional Options and Dropdown Items
ACT Name	Text	Read Only	

User Interaction

For more information, refer to *NovaSeq X Series Integration v1.2 Validation and Troubleshooting* on page 32.

Step 2: Make Bulk Pool (NovaSeq X Series Sequencing v1.1)

Samples are pooled in the Make Bulk Pool (NovaSeq X Series Sequencing v1.1) step. You can manually create working pools based on the final loading concentration required.

The following automations are configured on this step:

- Register Step Started
- Validate Analysis Configurations
- Calculate Volumes
- Set Next Step
- Register Pools & Register Step Completed

Register Step Started Automation

Automatically triggered on entry to the step, this automation registers the start time of the step. This automation completes this action by publishing messages to CLPA through ICA.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_started.groovy'
```

Validate Analysis Configurations Automation

Automatically triggered on exit of the Pooling screen, this automation performs analysis configuration validity and checks each pool for the following characteristics:

- Pooled samples are within the maximum configuration limit.
- Pooled samples have the same type of analysis (eg, Cloud or Local).
- Pooled samples that have the same secondary analysis also have the same analysis version (eg, v3.8.4) and analysis settings (eg, Map/Align Output Format = CRAM).

```
/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/novaseqxserie
s-automation.jar script:validate_physical_logical_configurations -i {stepURI:v2}
-u {username} -p {password} -l {compoundOutputFileLuid1} -m PerPool
script:validate_analysis_config -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} -m PerPool && /opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar
script:validate_same_analyte_udf_value_for_all_inputs -i {stepURI:v2} -u
{username} -p {password} -l {compoundOutputFileLuid1} -f 'Run Mode' -perPool
true
```

Calculate Volumes Automation

Automatically triggered when you select Calculate Volumes on the Record Details screen, this automation completes the following actions:

- Proceeds if the sample has molarity values.

```
if (!input.hasValue(::Molarity (nM)::)) { return; };
```

- Uses Number of Samples in Pool to calculate the volumes needed for the 2 nM intermediate library pools.

```
if (output.hasValue(::Number of Samples in Pool::)) { output.::Number of Samples
in Pool:: = output.::Number of Samples in Pool:: + 1; } else { output.::Number
of Samples in Pool:: = 1; }' -t true
```

- Uses the Bulk Pool Volume (µl) field and Number of Lanes to Sequence (x100) to calculate volumes needed for the 2 nM intermediate library pools.
- Copies the Final Loading Concentration (pM) and Flowcell Type from the step inputs to the step outputs.

```
output.::Bulk Pool Volume (ul):: = 100 * step.::Number of Lanes to Sequence::;
output.::NovaSeq X Flowcell Type:: = step.::Flowcell Type::; output.::Final
Loading Concentration (pM):: = step.::Final Loading Concentration (pM)::;
```

- Calculates the per sample volume required for each library to make a 2 nM intermediate library pool and sets the total sample volume to zero.

```
input.::Per Sample Volume (ul):: = 2 * output.::Bulk Pool Volume (ul):: /
input.::Molarity (nM):: / output.::Number of Samples in Pool::;output.::Total
Sample Volume (ul):: = 0;' -t true
```

- Calculates the adjusted per sample volume for the pools.

```
script:calculate_multipool_adjusted_per_sample_volume -t
```

- Uses the Total Sample Volume (µl) to calculate the RSB volume (µl) required to top up the pools that are needed to create the 2 nM intermediate library pools. The RSB volume is saved to the file in the Calculation File placeholder in the Files section.

```
script:evaluateDynamicExpression \
-exp 'if (output.hasValue(::Total Sample Volume (ul)::)) { output.::Total Sample Volume (ul):: = output.::Total Sample Volume (ul):: + input.::Adjusted Per Sample Volume (ul)::; } else { output.::Total Sample Volume (ul):: = input.::Adjusted Per Sample Volume (ul)::; }' -t true \
script:evaluateDynamicExpression \
-exp 'if (output.::Total Sample Volume (ul):: > output.::Bulk Pool Volume (ul)::) { output.::RSB Volume (ul):: = 0 } else { output.::RSB Volume (ul):: = output.::Bulk Pool Volume (ul):: - output.::Total Sample Volume (ul):: }' -t true \
```

- Uses the `NovaSeqXSeries_Bulk_Pool1.csv` and `NovaSeqXSeries_Bulk_Pool2.csv` template files to generate a single CSV file. This file contains information about the pools and the samples that they contain. The generated file is stored in the Calculation File placeholder in the Files section.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeqXSeries_Bulk_Pool1.csv -o 1.csv \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeqXSeries_Bulk_Pool2.csv -o 2.csv \
&& cat 1.csv 2.csv > {compoundOutputFileLuid0}.csv \
```

- Resets the Number of Samples in Pool, Total Sample Volume (µl), and Bulk Pool Volume (µl) to null before exiting the step.

```
-exp 'output.::Number of Samples in Pool:: = ::::; output.::Total Sample Volume (ul):: = ::::; output.::Bulk Pool Volume (ul):: = ::::;' -t true
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation completes the following actions:

- Proceeds only if the sample has a molarity value.
- Copies the Run Mode from input to output.
- Sets the next step for samples to ADVANCE, which advances them to the Dilute and Denature (NovaSeq X Series Sequencing v1.1) step.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} -log {compoundOutputFileLuid1} \
```

```
script:evaluateDynamicExpression -exp 'if (!input.hasValue(::Molarity (nM)::)){
return; }; output.::Run Mode:: = input.::Run Mode::; nextStep = ::ADVANCE::;'"
```

Register Pools & Register Step Completed Automation

Automatically triggered on exit of the step, this automation completes the following actions:

- Registers the pool and library information used by the Clarity LIMS workflow. Samples in the pool are assumed to have gone through library preparation.
- Registers the completion time of the step.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s
'com/illumina/upa/scripts/common/pooling.groovy' && /opt/gls/clarity/bin/java -
jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-
product-analytics-automation.jar script:executeUPAAutomationScript -i
{stepURI:v2} -u {username} -p {password} -s
'com/illumina/upa/scripts/common/step_completed.groovy'
```

This automation completes this action by publishing messages to CLPA through ICA.

Master Step Fields

The following table lists configuration details for the fields that are defined on the Make Bulk Pool (NovaSeq X Series Sequencing v1.1) step.

Table 5 Make Bulk Pool (NovaSeq X Series Sequencing v1.1) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Final Loading Concentration (pM)	Numeric Dropdown	Required Field Custom Entries	Dropdown Items • 90 • 140 • 150 • 160 • 180 Range from 0 Decimal places displayed: 0
Flowcell Type	Text	Required Field	Dropdown Items • 1.5B • 10B • 25B

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Field Name	Field Type	Options	Additional Options and Dropdown Items
Minimum Per Sample Volume (µl)	Numeric	Required Field	Decimal places displayed: 2 Default • 2
Number of Lanes to Sequence	Numeric	Required Field	Range: 1–10 Decimal places displayed: 0

Global Fields

The following table lists the global custom fields that are configured to display on the Make Bulk Pool (NovaSeq X Series Sequencing v1.1) step.

Table 6 Global Custom Fields Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
Final Loading Concentration (pM)	Numeric Dropdown	Required Field Custom Entries	Decimal places displayed: 0 Dropdown Items • 225 • 400
RSB Volume (µl)	Numeric	Read Only	Decimal places displayed: 2
NovaSeq X Flowcell Type	Text Dropdown	Required Field Read Only	Dropdown Items • 1.5B • 10B • 25B

User Interaction

For more information, refer to *NovaSeq X Series Integration v1.2 Validation and Troubleshooting* on page 32.

Step 3: Dilute and Denature (NovaSeq X Series Sequencing v1.1)

The Dilute and Denature (NovaSeq X Series Sequencing v1.1) step allows you to dilute pooled samples with the addition of RSB.

The following automations are configured on this step:

- Validate Inputs Flowcell Type and Register Step Started
- Calculate Volumes
- Set Next Step
- Register Step Completed

Validate Inputs Flowcell Type and Register Step Started

Automatically triggered on entry to the step, this automation completes the following actions:

- Checks that the specified flow cell type is valid.

  This script is required for the NovaSeq X Series Sequencing v1.1 workflow to function properly.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
script:evaluateDynamicExpression -h false -exp 'if (!input.hasValue(::NovaSeq X
Flowcell Type::)){fail(::Invalid Flowcell Type custom field value. Please make
sure all input samples have a value assigned for Flowcell Type custom
field.::)}' -log {compoundOutputFileLuid1}
```

- Registers the start time of the step by publishing messages to CLPA through ICA.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s
'com/illumina/upa/scripts/common/step_started.groovy'
```

Calculate Volumes Automation

Automatically triggered when you select Calculate Volumes on the Record Details screen, this automation completes the following actions based on the NovaSeq X Flowcell Type selected:

- Sets the NaOH Volume (µl) and TT2 Volume (µl) field values.
- Computes the BP Aliquot Volume (µl) and RSB Volume (µl) required for the dilution of pools to the required final loading concentration.
- Sets the PhiX Volume (µl) and PhiX Concentration (µl) if there is a PhiX spike-in.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} -
log {compoundOutputFileLuid1} \
script:evaluateDynamicExpression \
-exp '
if (input.::NovaSeq X Flowcell Type:: == ::10B:: || input.::NovaSeq X Flowcell
Type:: == ::1.5B::) {
```


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```
output.::NaOH Volume (ul):: = 8.5;
output.::TT2 Volume (ul):: = 127.5;
output.::BP Aliquot Volume (ul):: = input.::Final Loading Concentration (pM):: *
170 / (2 * 1000);
output.::RSB Volume (ul):: = 34 - output.::BP Aliquot Volume (ul)::;
if (step.::1-2% PhiX Spike-In::) { output.::PhiX Volume (ul):: = 1;
output.::PhiX Concentration (pM):: = 300; }
else { output.::PhiX Volume (ul):: = ::::; output.::PhiX Concentration (pM):: =
::::; };
} else {
output.::NaOH Volume (ul):: = 14;
output.::TT2 Volume (ul):: = 210;
output.::BP Aliquot Volume (ul):: = input.::Final Loading Concentration (pM):: *
280 / (2 * 1000);
output.::RSB Volume (ul):: = 56 - output.::BP Aliquot Volume (ul)::;
if (step.::1-2% PhiX Spike-In::) { output.::PhiX Volume (ul):: = 1.6;
output.::PhiX Concentration (pM):: = 300; }
else { output.::PhiX Volume (ul):: = ::::; output.::PhiX Concentration (pM):: =
::::; };
}'
```

- Generates a single CSV file that contains information about the reagents volume required to dilute the working pools. The generated file is stored in the Calculation File placeholder in the Files section.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeqXSeries_Dilute_
Denature_Calculate_Volumes.csv -o {compoundOutputFileLuid0}.csv -q true -
destLIMSID {compoundOutputFileLuid0} -l {compoundOutputFileLuid1} \
&& echo; echo 'Calculate Volumes completed successfully.'"
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation completes the following actions:

- Copies the Run Mode from input to output.
- Sets the next step for samples to ADVANCE, advancing them to the Load to Library Tube Strip (NovaSeq X Series Sequencing v1.1) step.

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```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
script:evaluateDynamicExpression -t false -h false -exp 'output.::Run Mode:: =
input.::Run Mode::; nextStep = ::ADVANCE::' -log {compoundOutputFileLuid1}
```

Register Step Completed Automation

Automatically triggered on exit of the step, this automation registers the completion time of the step by publishing messages to CLPA through ICA.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s
'com/illumina/upa/scripts/common/step_completed.groovy'
```

Global Fields

The following table lists the global custom fields that are configured to display on the Dilute and Denature (NovaSeq X Series Sequencing v1.1) step.

Table 7 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
BP Aliquot Volume (µl)	Numeric	Read Only	Decimal places displayed = 0
NaOH Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
RSB Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
TT2 Volume (µl)	Numeric	Read Only	Decimal places displayed = 2

User Interaction

For more information, refer to *NovaSeq X Series Integration v1.2 Validation and Troubleshooting* on page 32.

Step 4: Load to Library Tube Strip (NovaSeq X Series Sequencing v1.1)

The Load to Library Tube Strip (NovaSeq X Series Sequencing v1.1) step allows you to scan the library tube strip barcode into Clarity LIMS. Then, you can place the working pools into the library tube strip used in the NovaSeq X Series run. This step also does the following actions:

- Validates the run setup and analysis information.
- Generates the sample sheet file.
- Creates a planned run on ICA, depending on the selected run mode.

The following automations are configured on this step:

- Validate Flowcell Inputs and Validate Analysis Configurations and Register Step Started
- Validate Library Tube Strip Barcode
- Validate Run Setup and Create Planned Run
- Set Next Step
- Register Step Completed

Validate Flowcell Inputs and Validate Analysis Configurations and Register Step Started Automation

Automatically triggered on entry to the step, this automation completes the following actions:

- Checks that the selected destination container types on the Load to Library Tube Strip step matches the selected flow cell type of the samples. The 1.5B flow cell is compatible with the library 2-tube strip and the 10B and 25B flow cells are compatible with the library 8-tube strip.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} \
script:validate_same_udf_value_for_analytes -f 'Run Mode' -f 'NovaSeq X Flowcell
Type' \
script:validate_selected_container \
-fn 'NovaSeq X Flowcell Type' -fv '1.5B' -ct 'Library 2-tube Strip' \
-fn 'NovaSeq X Flowcell Type' -fv '10B' -ct 'Library 8-tube Strip' \
-fn 'NovaSeq X Flowcell Type' -fv '25B' -ct 'Library 8-tube Strip'
```

- Performs basic checks on the secondary analysis configuration of the samples in the same planned run. The following checks are included in this script:
  - Secondary analysis configuration of samples in a planned run is within maximum configuration limit.
  - Samples in a planned run have the same type of analysis (eg, Cloud or Local).
  - Samples in the same pools that have the same secondary analysis (eg, Illumina DRAGEN Enrichment) also have the same analysis settings (eg, Map/Align Output Format = CRAM) and the same analysis version (eg, v3.8.4).

    This script is required for the NovaSeq X Series Sequencing v1.1 workflow to function properly.

```
/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/novaseqxseries-automation.jar -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -m PerRun script:validate_analysis_config script:validate_physical_logical_configurations
```

- Registers the start time of the step by publishing messages to CLPA through ICA.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s 'com/illumina/upa/scripts/common/step_started.groovy'
```

Validate Library Tube Strip Barcode Automation

Automatically triggered on the Placement screen, this automation validates the library tube strip barcode to make sure that it conforms to the barcode mask LC[0-9]{7}-LC1 for the library 8-tube strip and LC[0-9]{7}-L[A-Z]2 for the library 2-tube strip.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:validate_output_containers -l {compoundOutputFileLuid1} -r 'Library 8-tube Strip:LC[0-9]{7}-L[A-Z]1' -r 'Library 2-tube Strip:LC[0-9]{7}-L[A-Z]2' -max 1
```

Validate Run Setup and Create Planned Run Automation

Automatically triggered after a selection on the Record Details screen, this automation completes the following actions:

- Validates the parameters entered on the Record Details screen. These parameters are used to set up the run, generate the sample sheet file, and create the planned run in ICA, depending on the run mode.
  - Run Name can only contain alphanumeric, dash, underscore, or period characters. Do not use spaces.

```
if (!step.::Run Name::.matches(::[a-zA-Z0-9-_]+[a-zA-Z0-9-_. ]*::)) { fail(::Run Name contains prohibited characters. Please check to make sure Run Name only contains alphanumeric characters, spaces, dashes and underscores. Run Name must start with alphanumeric, a dash or an underscore::); }
```

  - Run Name cannot exceed 255 characters.

```
if (step.::Run Name::.length() > 255) { fail(::Run Name shall not exceed 255 characters.::); };
```

- Checks the Index 1 Cycles and Index 2 Cycles field values. If Index 2 Cycles is greater than 0, the Index 1 Cycles value must be greater than 0 or an error can occur.

```
if (step.::Index 2 Cycles:: > 0 && step.::Index 1 Cycles:: == 0) { fail(::Index
1 Cycles cannot be zero if Index 2 Cycles is non-zero::); };
```

- Generates the sample sheet and creates the planned run in ICA. The sample sheet is attached to the step.

```
/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/novaseqxserie
s-automation.jar script:generate_sample_sheet -i {stepURI:v2} -u {username} -p
{password} -c {compoundOutputFileLuid0} -l {compoundOutputFileLuid1}
```

There is a backend validation of the sample sheet content in ICA. This validation makes sure that the sample sheet is valid to set up a Local mode run and that it is ready for use with DRAGEN applications.

For more information on sample name character restrictions, refer to *NovaSeq X Series Integration v1.2 Validation and Troubleshooting* on page 32.

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation completes the following actions:

- Copies the Run Mode from input to output.
- Sets the next step for samples to ADVANCE, advancing them to the AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.1) step.

```
output.::Run Mode:: = input.::Run Mode::; nextStep = ::ADVANCE::
```

Register Step Completed Automation

Automatically triggered on exit of the step, this automation registers the completion time of the step by publishing messages to CLPA through ICA.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s
'com/illumina/upa/scripts/common/step_completed.groovy'
```

Master Step Fields

The following table shows the master step fields that are configured on the Load to Library Tube Strip (NovaSeq X Series Sequencing v1.1) step. These fields are required for sample sheet generation and planned run creation in ICA.

Table 8 Load to Library Tube Strip (NovaSeq X Series Sequencing v1.1) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Cloud Run ID	Text	Read Only	Hidden Used for creating a planned run in ICA. Do not remove, rename, or modify this field as it can cause the integration to break.
Index 1 Cycles	Numeric Dropdown	Required Field Custom Entries	Range = 0–20 Presets • 0 • 6 • 8
Index 2 Cycles	Numeric Dropdown	Required Field Custom Entries	Range = 0–20 Presets • 0 • 6 • 8
Read 1 Cycles	Numeric Dropdown	Required Field Custom Entries	Range = 1–251 Presets • 51 • 101 • 151
Read 2 Cycles	Numeric Dropdown	Required Field Custom Entries	Range = 0–251 Presets • 51 • 101 • 151
Run Mode	Text Dropdown	Required Field	Presets • Local • Cloud
Run Name	Text	Required Field	

User Interaction

For more information, refer to *NovaSeq X Series Integration v1.2 Validation and Troubleshooting* on page 32.

Step 5: AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.1)

The AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.1) step is fully automated. The integration starts the step, adds samples to the Ice Bucket, and completes the step. Data from the run is parsed back to Clarity LIMS. In this step, pooled samples in the library tube strip are sequenced on the NovaSeq X Series instrument.

> Do not add samples to the Ice Bucket or start and complete the AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.1) step. The integration does this automatically.

The following automations are configured on this step:

- Register Step Started & Register NovaSeq X Run & Register NovaSeq X Run Association & Register NovaSeq X RunStarted
- Register NovaSeq X RunCompleted & Register Step Completed

Register Step Started & Register NovaSeq X Run & Register NovaSeq X Run Association & Register NovaSeq X RunStarted Automation

Automatically triggered at the beginning of the step, this automation completes the following actions:

- Registers the start time of the step.
- Registers the NovaSeq X Series sequencing run configuration.
- Registers the association of the sequencing run with the samples by linking the Instrument Run ID with the sample ID.
- Registers the time that the sequencing run started.

This automation completes these actions by publishing messages to CLPA through ICA.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s
'com/illumina/upa/scripts/common/step_started.groovy' &&
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s
'com/illumina/upa/scripts/novaseqxseries/register_novaseqxseries_run.groovy' &&
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s
'com/illumina/upa/scripts/novaseqxseries/novaseqxseries_associate_seqrun.groovy'
```

```
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s
'com/illumina/upa/scripts/novaseqxseries/novaseqxseries_seqrun_started.groovy'
```

Register NovaSeq X RunCompleted & Register Step Completed Automation

Automatically triggered on exit of the step, this automation completes the following actions:

- Registers the sequencing run completion time, run status, and run metrics. This script requires the Cloud Run ID custom field from the Load to Library Tube Strip (NovaSeq X Series Sequencing v1.1) step.
- Registers the completion time of the step.

This automation completes these actions by publishing messages to CLPA through ICA.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s
'com/illumina/upa/scripts/novaseqxseries/novaseqxseries_seqrun_completed.groovy'
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s
'com/illumina/upa/scripts/common/step_completed.groovy'
```

Master Step Fields

The following tables show the master step fields that are configured on the AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.1) step.

Table 9 Clarity LIMS Master Step Field Information

Field Name	Source File	Corresponding Field in Source File	Additional Information
Current Cycle	`RunCompletionStatus.xml`	CompletedReads	Visible on Record Details screen. Calculated based on the CompletedReads field.

Field Name	Source File	Corresponding Field in Source File	Additional Information
Current Read	`RunCompletionStatus.xml` `RunParameters.xml`	CompletedReads PlannedReads	Visible on Record Details screen. Calculated based on the CompletedReads field in `RunCompletionStatus.xml` and PlannedReads field in `RunParameters.xml`.
Flow Cell Expiration Date	`RunParameters.xml`	ExpirationDate	Visible on Record Details screen. Corresponds to the value in the ExpirationDate field under the ConsumableInfo field for FlowCell Type.
Flow Cell ID	`RunParameters.xml`	SerialNumber	Visible on Record Details screen. Corresponds to the value in the SerialNumber field under the ConsumableInfo field for FlowCell Type.
Flow Cell Lot Number	`RunParameters.xml`	LotNumber	Visible on Record Details screen. Corresponds to the value in the LotNumber field under the ConsumableInfo field for FlowCell Type.
Flow Cell Part Number	`RunParameters.xml`	PartNumber	Visible on Record Details screen. Corresponds to the value of the PartNumber field under the ConsumableInfo field for FlowCell Type.
Flow Cell Side	`RunParameters.xml`	Side	Visible on Record Details screen.
Flow Cell Type	`RunParameters.xml`	FlowCellType	Visible on Record Details screen.

Field Name	Source File	Corresponding Field in Source File	Additional Information
Instrument Control Software Version	`RunParameters.xml`	SystemSuiteVersion	Visible on Record Details screen.
Instrument ID	`RunParameters.xml`	InstrumentSerialNumber	Visible on Record Details screen.
Instrument Type	`RunParameters.xml`	InstrumentType	Visible on Record Details screen.
Output Folder	`RunParameters.xml`	OutputFolder	Visible on Record Details screen.
Library Tube Strip Barcode	`RunParameters.xml`	SerialNumber	Visible on Record Details screen. Corresponds to the value of the SerialNumber field under the ConsumableInfo field for SampleTube Type.
Library Tube Lot Number	`RunParameters.xml`	LotNumber	Hidden Do not remove, rename, or modify this field as it can cause the integration to break. Corresponds to the value of the LotNumber field under the ConsumableInfo field for SampleTube Type.
Run End Time	`RunCompletionStatus.xml`	RunEndTime	Hidden Do not remove, rename, or modify this field as it can cause the integration to break.
Run Name	`RunParameters.xml`	ExperimentName	Visible on Record Details screen.
RTA Version	Not used	Not used	Hidden

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Table 10 Other Clarity LIMS Master Step Fields

Field Name	Value	Additional Information
Run Status	Presets • RunStarted • RunCompletedSuccessfully • RunAbortedByUser • RunErroredOut Set by the SIS integration service.	Visible on Record Details screen.
Sequencing Log	Set by the SIS integration service during the sequencing run.	Visible on Record Details screen.
BaseSpace Run ID	Received from the SQS message in the SIS integration service.	Hidden Do not remove, rename, or modify this field as it can cause the integration to break.
ICA Project ID	Received from the SQS message in the SIS integration service.	Hidden Do not remove, rename, or modify this field as it can cause the integration to break.
Instrument Run ID	Received from the SQS message in the SIS integration service.	Hidden Do not remove, rename, or modify this field as it can cause the integration to break.
Run Start Time	Received from the SQS message in the SIS integration service.	Hidden Do not remove, rename, or modify this field as it can cause the integration to break.

Global Fields

The following global custom fields are used to capture the run metrics in Clarity LIMS:

- % Bases >=Q30 R1
- % Bases >=Q30 R2

- % Error Rate R1
- % Error Rate R2
- Yield (Gb) R1
- Yield (Gb) R2
- Reads PF R1
- Reads PF R2
- %PF R1
- %PF R2
- % Aligned R1
- % Aligned R2
- % Phasing R1
- % Phasing R2
- % PrePhasing R1
- % PrePhasing R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- Cluster Density R1
- Cluster Density R2

At the end of the step, the pools of samples are automatically removed from the step. The step completes automatically when Run Status is RunCompletedSuccessfully.

Step 6: AUTOMATED - Analysis Run (NovaSeq X Series Sequencing v1.1)

The AUTOMATED - Analysis Run (NovaSeq X Series Sequencing v1.1) step is fully automated. The integration starts and completes the step automatically. Data from the analysis is parsed back to Clarity LIMS. In this step, the secondary analysis configured using the ACT is performed in ICA. There are no automations associated with this step.

Do not add samples to the Ice Bucket or start and complete the AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.1) step. The integration does this automatically.

Master Step Fields

The following tables show the master step fields that are configured on the AUTOMATED - Analysis Run (NovaSeq X Series Sequencing v1.1) step.

Do not modify the names of the master step fields. Modifications cause the integration to break.

Table 11 AUTOMATED - Analysis Run (NovaSeq X Series Sequencing v1.1) Master Step Field Information

Field Name	Source File	Additional Information
Analysis Status	Presets • Started • Completed • Failed • NeedsAttention • Stopped • TimedOut • CompletedWithErrors Set by the SIS integration service.	Visible on Record Details screen.
Analysis Result Location	Received from the SQS message in the SIS integration service.	Visible on Record Details screen.
Library Tube Strip Barcode	Identified by the SIS integration service using information from `RunParameters.xml`.	Hidden

How the Integration Works

The following information summarizes how the NovaSeq X Series integration works.

- After the Validate Run Setup and Create Planned Run automation is triggered on the Load to Library Tube Strip (NovaSeq X Series Sequencing v1.1) step, the run parameters entered in the Run Details screen are sent to ICA. The analysis configuration parameters from the selected ACTs are also sent to ICA. ICA validates the run and analysis configuration. If the validation fails, ICA sends an error message to Clarity LIMS. If the validation passes, ICA generates the sample sheet and sends it back to Clarity LIMS. ICA also creates the planned run based on the selected run mode (eg, Local or Cloud).
  - For the Local run mode, the sample sheet is generated and stored in Clarity LIMS. This sample sheet contains the run and analysis configuration information required to start the run on the NovaSeq X Series instrument.
  - For the Cloud run mode, the planned run is created in ICA. The planned run contains the run and analysis configuration information required to start the run on the instrument. The sample sheet is generated and stored in Clarity LIMS for reference purposes.
- When the sequencing run starts on the instrument, the NovaSeq X Series Control Software notifies BaseSpace Sequence Hub. BaseSpace Sequence Hub monitors the run and uses the Stratus Integration Service (SIS) to notify Clarity LIMS. The events are processed and the integration

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service retrieves the run information from BaseSpace Sequence Hub. This information is used to populate the custom fields in the AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.1) step.

- Other run events follow the same information flow. When sequencing is complete, the control software uploads the sequencing run data (primary metrics) and associated files to ICA. Then, Clarity LIMS retrieves the primary metrics and uses them to populate the fields in the Sample Details table (eg, % Error Rate R1). The custom fields (eg, Run Status, Current Read, and so on) on the AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.1) step are updated using the run related information. If the sequencing run is successfully completed, the step automatically completes.
- The integration tracks the analysis events and results in the AUTOMATED - Analysis Run (NovaSeq X Series Sequencing v1.1) step. You must log in to BaseSpace Sequence Hub to retrieve the cloud analysis results. For local analysis, the secondary analysis results are in the external storage configured in Illumina Run Manager. The external storage information is found in the External Storage for Analysis Results configuration settings in Illumina Run Manager.
- If a planned run with the same sample name and project name (case-insensitive) has been created previously in ICA, the sample sheet generated from the Validate Run Setup and Create Planned Run automation can reflect the original case of the previous sample name. This can cause validation errors for analysis configurations with sample-level settings. To resolve this issue, change the sample name or the project name on Clarity LIMS and run the automation again.

Start a Sequencing Run on NovaSeq X Series Instrument

The following sections outline the steps to start a Clarity LIMS cloud or local run on the NovaSeq X Series instrument.

Run Mode Configuration

1. In the NovaSeq X Series Control Software, log in to the instrument using an account that has permissions for configuring settings.
2. On the menu, select Settings, then select Cloud settings.
3. In Run Settings, select the Cloud run storage checkbox.
4. For Hosting location, select the applicable location from the drop-down options.
5. [Optional] For Private domain name, enter the domain name according to the workgroup that was configured with the NovaSeq X Series integration package.
6. Select Save.

Start a Sequencing Run in Local Run or Cloud Run Modes

Start a Sequencing Run in Local Run Mode

1. Log in with the BaseSpace Sequence Hub account and select the workgroup used during the NovaSeq X Series integration package installation.
2. Import the sample sheet created in the Load to Library Tube Strip (NovaSeq X Series Sequencing v1.1) step. Refer to information on creating a local run in the *NovaSeq X Plus Product Documentation* on the NovaSeq X Plus Sequencing System support site.
3. When the sample sheet is selected, the contents are validated. After the validation passes, select Next.
4. Select Save As Planned.
   Run and analysis data is parsed and auto-populates in the list of runs. For more information on planning a NovaSeq X Series run, refer to the Plan a NovaSeq X Series Run section of the BaseSpace Sequence Hub Online Help at help.basespace.illumina.com and the *NovaSeq X Plus Product Documentation* on the NovaSeq X Plus Sequencing System support site.
5. Start the run. Refer to information on starting a planned run in the *NovaSeq X Plus Product Documentation* on the NovaSeq X Plus Sequencing System support site.

Start a Sequencing Run in Cloud Run Mode

To start a sequencing run in cloud run mode, refer to information on starting a planned run in the *NovaSeq X Plus Product Documentation* on the NovaSeq X Plus Sequencing System support site.

Components Installed

The following sections describe the components (files, properties, reagent categories / label groups, reagent kits, and containers) that are installed by default as part of this integration.

Reagent Kits

- Buffer Cartridge
- Lyophilization Cartridge
- NaOH
- Reagent Cartridge
- Resuspension Buffer (RSB)
- TT2

Container Types

- Library 2-Tube Strip
- Library 8-Tube Strip
- Tube

This integration supports the library 8-tube strip with the barcode in the LC[0-9]{7}-L[A-Z]1 format. The integration also supports the library 2-tube strip with the barcode in the LC[0-9]{7}-L[A-Z]2 format.

Control Types

- PhiX v3

Rules and Constraints

- The workflow configuration contains several validation checks. To make sure that the calculations work properly, it is important that you do not disable any of this validation logic. The validation checks determine the following information:
  - Which samples, and how many, can enter each step together.
  - Which samples, and how many, can be pooled together.
- All submitted samples must have an associated secondary analysis that is configured using the analysis configuration template (ACT). The ACT must be configured on BaseSpace Sequence Hub before starting the Assign Analysis Configuration Template step. The ACT names must be unique.
- The library tube strip barcode must be unique. There must not be multiple library tube strip containers with the same name in the system.
- Reagent labels, or indexes, must be unique.
- One library pool can only contain one library or control with no label/index.
- The AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.1) and AUTOMATED - Analysis Run (NovaSeq X Series Sequencing v1.1) steps must not be manually started or completed. These steps are fully automated and the sequencing service does not update samples correctly if they have been manually started.
- For the automated run to start successfully, you must select Validate Run Setup and Create Planned Run in the Load to Library Tube step.

NovaSeq X Series Integration v1.2 Validation and Troubleshooting

This section explains how to validate the installation of the Illumina NovaSeq X Series Integration Package v1.2.

The validation process involves the following actions:

- Running samples through the Library Prep Validation v2.3.2 workflow.
  - The workflow contains a single-step protocol that models the library prep required to produce libraries tagged with index sequences. At the end of the step, these libraries are routed to NovaSeq X Series Sequencing v1.1 workflow.
- Running libraries through the NovaSeq X Series Sequencing v1.1 workflow validates the following items:
  - Successful sequential step advancement of samples through the steps of the workflow.

- Automatic validation of run setup information before sample sheet generation or planned run creation.
- Automated sample sheet generation. This sample sheet is used to start the sequencing run on the NovaSeq X Series Control Software through the Local run mode.
- Automated creation of a planned run in Illumina Connected Analytics (ICA). The control software retrieves the planned run. The run is started through the Cloud run mode.
- Automated tracking of the NovaSeq X Series sequencing run and parsing of run statistics from BaseSpace Sequence Hub to Clarity LIMS.
- Automated tracking of the NovaSeq X Series analysis run and high level analysis summary from BaseSpace Sequence Hub to Clarity LIMS.

The validation steps assume that the following conditions have been met:

- The NovaSeq X Series Integration v1.2 has a valid BaseSpace Sequence Hub account with an Enterprise or Professional subscription.
- NovaSeq X Series Integration Package v1.2 is installed and you have imported the default Clarity LIMS configuration.
- Analysis configuration templates (ACTs) are created in BaseSpace Sequence Hub (BaseSpace Sequence Hub) for your run. Make sure that the index adapter kit label group is created in Clarity LIMS before selecting it in the ACT. This same label group is used in the Run Library Prep Validation v2.3.2 step. For more information on creating a reagent label group, refer to *Add and Configure Labels and Label Groups* in the Clarity LIMS Core Documentation. For the adapter sequences for your library prep kits, refer to *Illumina Adapter Sequences*.

Analysis Configuration Template Creation in BaseSpace Sequence Hub

You must create the Analysis Configuration Templates (ACTs) that are required for configuring secondary analysis in the NovaSeq X Series Sequencing v1.1 workflows. Create and delete ACTs in BaseSpace Sequence Hub. For instructions, refer to the *BaseSpace Sequence Hub Online Help* on the Illumina support site.

Activate Workflow, Create Project, Add and Assign Samples

The following steps set up Clarity LIMS in preparation for running samples through the Library Prep Validation v2.3.2 and NovaSeq X Series Sequencing v1.1 workflows.

Sample and library names must use only alphanumeric, dash, or underscore characters. Invalid characters can cause a sample sheet validation failure in the Load to Library Tube Strip step.

1. On the Configuration tab, under Workflows, activate both the Library Prep Validation v2.3.2 and NovaSeq X Series Sequencing v1.1 workflows.
2. On the Projects and Samples screen, create a project and add samples to it.
3. Assign the samples to the Library Prep Validation workflow.

Library Prep Protocol: Library Prep Validation v2.3.2

This single-step protocol models the library prep required to produce libraries tagged with index sequences that are ready for the NovaSeq X Series Sequencing v1.1 workflow.

Run Library Prep Validation v2.3.2

1. In Lab View, locate the Library Prep Validation v2.3.2 protocol.
   Samples are queued for the Library Prep Validation v2.3.2 step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, under Label Options, select the label groups that you want to use.
   The label group, or index adapter kit, selected must be the same as the Index Adapter Kit selected in the ACT that is being used.

   TruSeq HT Adapters v2 (D7-D5) is the default label group in the Library Prep Validation v2.3.2. For other reagent labels, you can manually create them. For more information, refer to *Add and Configure Labels and Label Groups* in the *Clarity LIMS Core Functionality* documentation.

   The adapter sequences from Illumina library prep kits, refer to Illumina Adapter Sequences.
4. Select Begin Work.
5. On the Placement screen, complete the following actions:
   a. In the Placed Samples area on the right, in the Container field, scan or enter the barcode of the destination container.
   b. Select the samples from the Samples to be Placed area on the left, and drag them to the container wells on the right.
   c. Select Add Labels.
6. On the Add Labels screen, the Labels to Add list contains labels from the label group selected in 3.
   a. Select labels on the left, and drag them to the container on the right to assign them to placed samples.
   b. Select Record Details.
7. On the Record Details screen, complete the following actions:
   a. In the Sample Details table, enter values for Molarity (nM). Type values individually or copy and paste to enter multiple values from a spreadsheet.

      The Molarity (nM) values are used in the Calculate Volume automation script in the Make Bulk Pool (NovaSeq X Series Sequencing v1.1) step. If these values are not entered, the automation script does not work properly.

   b. The Sequencing Instrument column indicates the instrument to be used for the sequencing run. Select NovaSeq X Series.
   c. Populate the fields in the Sequencing Instrument column using one of the following methods:
      - To populate fields individually for each sample, select from the drop-down list in each row.

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- To populate all fields at one time, copy and paste from a spreadsheet.
- Alternatively, select inside the table and press Ctrl + A to select all rows. At the top of the table, select Sequencing Instrument, and then select NovaSeq X Series from the adjacent drop-down list. Select Apply.

If working with many samples, copy and paste to insert multiple values from a spreadsheet as follows.

a. Select Copy Samples to copy the sample information from Clarity LIMS to the Clipboard.
b. Open a blank Excel document and paste the contents of the clipboard into the spreadsheet.
c. In the spreadsheet, enter the Molarity (nM) and Sequencing Instrument values. Select the entire spreadsheet including the headers (Ctrl + A), and copy (Ctrl + C) the information to the Clipboard.
d. Within Clarity LIMS, select the Paste Samples button. Paste the contents of the clipboard (Ctrl + V) into the Update Table window and then select Update to paste the contents into the Sample Details table.

8. Select Next Steps.
   This selection triggers the Set Next Steps automation, which sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step.
9. Select Finish Step.
   On exit from the step, the Routing Script automation is triggered. This automation assigns samples to the first step of the NovaSeq X Series Sequencing v1.1 workflow, Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.1).

Protocol: NovaSeq X Series Sequencing v1.1

The NovaSeq X Series Sequencing v1.1 protocol consists of the following steps:

- Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.1)
- Make Bulk Pool (NovaSeq X Series Sequencing v1.1)
- Dilute and Denature (NovaSeq X Series Sequencing v1.1)
- Load To Library Tube Strip (NovaSeq X Series Sequencing v1.1)
- AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.1)
- AUTOMATED - Analysis Run (NovaSeq X Series Sequencing v1.1)

Each step contains a script to register the start time (upon step entry) and the time that the step is completed (upon step exit). This information is published to CLPA through the ICA LINK service. These scripts are used only for CLPA support and can be incorporated as part of an automation that performs other functions.

Step 1: Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.1)

1. In Lab View, locate the NovaSeq X Series Sequencing v1.1 protocol. The samples are queued for the Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.1) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work to start the Validate Sample Names and Retrieve Analysis Configuration Template List and Register Step Started automation.
   The automation checks that the sample names do not use restricted characters and are within character limits and retrieves the list of ACTs that you can select. The automation also registers the start time of the step by publishing messages to CLPA through the ICA LINK service. The part of the script for start time registration is used only for CLPA support.
4. Select the applicable ACT to assign to the samples. The index adapter kit specified by the ACT must correspond with the label group used in *Library Prep Protocol: Library Prep Validation v2.3.2* on page 34.
5. [Optional] In Step Details, select Retrieve ACT Information to trigger the Retrieve ACT Information automation.
   This automation retrieves ACT information (eg, Library Prep Kit, Index Adapter Kit, Reference Genome, and so on) and populates the fields in Clarity LIMS. These details are saved to the `ACTMetadata.csv` file that you can download. If you are not sure of the analysis configuration before starting the sequencing and analysis run, refer to the details in the file.
6. Select Next Steps to assign the ACT to the samples.
   This action triggers the Validate Reagent Labels and Apply Selected ACT to Samples and Set Next Step automation. This automation validates that the indexes applied to the libraries are valid for the selected ACT and assigns samples to it. All samples added to the Ice Bucket in 2 are assigned to this ACT.
7. Select Finish Step.

Step 2: Make Bulk Pool (NovaSeq X Series Sequencing v1.1)

1. In Lab View, locate the NovaSeq X Series Sequencing v1.1 protocol. The samples are queued for the Make Bulk Pool (NovaSeq X Series Sequencing v1.1) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
   You can also add control samples by selecting them from the Available control samples drop-down list and selecting Add.
3. On the Ice Bucket screen, select Begin Work.
4. Create a pool of samples as follows.
   a. On the Pooling screen, create a pool by dragging samples into the Pool Creator.
      If a control sample was added, it appears under the Sample List and can be pulled into the pool that needs the control.

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b. Enter a name for your pool or accept the default name (Pool #1).

c. [Optional] If multiple pools are required, select the plus sign (+) next to Pool Creator to create a pool.

d. [Optional] To remove a pool, select the X in the top right corner of the pool.

e. Select Record Details to trigger the Validate Analysis Configurations automation.
This automation performs the following checks on the analysis configuration for each pool:

- Pooled samples are within the maximum configuration limit.
- Pooled samples have the sample type of analysis (Cloud or Local).
- Pooled samples that have the same secondary analysis also have the same analysis version and settings.

5. On the Record Details screen, navigate to the Reagent Lot Tracking section to track the lot information used in the step.
6. [Optional] Create a new lot. For more information, refer to *Add and Configure Reagent Kits and Lots* in the Clarity LIMS LIMS Core Functionality documentation.
7. In the Step Details area, complete the following required fields:
   - Number of Lanes to Sequence—Used in volume calculations, to make sure that the volumes are sufficient for the number of times the pool is sequenced. This field is applied to all pools in the step.
   - Final Loading Concentration (pM)—The final loading concentration of the pool in the flow cell.
   - Minimum Per Sample Volume (µl)—The minimum volume for each sample. This field is prepopulated with the configured default value (2 µl) and can be edited. If the per sample volume is below the value set in this field, the Calculate Volumes script applies a rounding factor to affected samples so that the volume reaches the minimum volume.
   - Flowcell Type—The flow cell type that the run uses. This selection affects the computation of volumes in the Calculate Volumes automation that generates the calculation file. Select from the following options:
     - 1.5B
     - 10B
     - 25B
8. Select Calculate Volumes to trigger the Calculate Volumes automation.
   This automation calculates the volumes required for each library to form a pool that has the concentration and volume specified in the Step Details field. It also generates the calculation file in a CSV format and attaches it to the step. Select the file name to download it.
9. In the Sample Details table, select the pool next to the sample name to view details on the pool composition.
10. Select Next Steps to trigger the Set Next Step automation.

This automation sets the next step for samples to ADVANCE, which moves them to the Dilute and Denature (NovaSeq X Series Sequencing v1.1) step.

11. Select Finish Step.

Step 3: Dilute and Denature (NovaSeq X Series Sequencing v1.1)

1. In Lab View, locate the NovaSeq X Series Sequencing v1.1 protocol. The pool of samples queued for the Dilute and Denature (NovaSeq X Series Sequencing v1.1) displays.
2. Add the pool to the Ice Bucket and select View Ice Bucket.
3. [Optional] On the Ice Bucket screen, set the number of derivatives to create (placed into the library tube strip) and select Begin Work.
   - On entry to the step, the Validate Inputs Flowcell Type and Register Step Started automation is triggered. This automation makes sure that the configured flow cell type is valid. The automation also registers the start time of the step by publishing messages to CLPA through the ICA LINK service. The portion of the script used for start time registration is used only for CLPA support.
   - If PhiX is used for the run, navigate to the Record Details screen and set 1–2% PhiX Spike-In to Yes. Select Calculate Volumes to trigger the Calculate Volume automation. This automation sets the following values:
     - BP Aliquot Volume (µl)
     - RSB Volume (µl)
     - NaOH Volume (µl)
     - TT2 Volume (µl)
     - PhiX Volume (µl) and Concentration (pM) (if there is a PhiX spike-in)
     - The automation also generates the calculation file (CSV) and attaches it to the step. This file contains information about the volume of RSB, NaOH, and TT2 to add per working pool. If there is a PhiX spike-in, the file also contains information on the PhiX volume and concentration.
4. On the Record Details screen, the Reagent Lot Tracking section tracks the NaOH, Resuspension Buffer, and TT2 reagents used in the step. To add and activate reagent lots, refer to *Add and Configure Reagent Kits and Lots* in the Clarity LIMS LIMS Core Functionality documentation.
5. On the Record Details screen, perform the following actions:
   a. In the Reagent Lot Tracking section, select from the active lots displayed in each drop-down list.
   b. In the Sample Details table, make sure that the BP aliquot, RSB, NaOH, and TT2 reagent volume values are populated. The script sets these values and they cannot be edited.
   c. [Optional] In the Sample Details table, select the pool icon to view details of the working pool composition.

6. In the Files area, select the Calculation File (CSV) to open it and view details on the volumes for each reagent and sample pool used for the dilute and denature process.
7. Select Next Steps.
   On the Assign Next Step screen, the next step is already set to Load to Library Tube Strip (NovaSeq X Series Sequencing v1.1).
8. Select Finish Step.

Step 4: Load to Library Tube Strip (NovaSeq X Series Sequencing v1.1)

1. In Lab View, locate the NovaSeq X Series Sequencing v1.1 protocol. The pool of samples are queued for the Load to Library Tube Strip (NovaSeq X Series Sequencing v1.1) step.
2. Add the pools to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select one of the following destination container types:
   - Library 8-tube Strip
   - Library 2-tube Strip
4. Select Begin Work to trigger the Validate Flowcell Inputs and Validate Analysis Configurations and Register Step Started automations.
   - The Validate Flowcell Inputs automation ensures that the correct container is selected for the flow cell type and that the number of inputs is the same as the number of available wells on the library tube strip. The Validate Analysis Configurations automation is similar to the automation used in the Make Bulk Pool step.
   - The Register Step Started automation also registers the start time of the step by publishing messages to CLPA through the ICA LINK service. The portion of the script used for start time registration is used only for CLPA support.
5. To use multiple ACTs in a single run, repeat steps 1–4 for each ACT.
   The pool of samples for each ACT is then queued for the Load to Library Tube Strip (NovaSeq X Series Sequencing v1.1) step to be added to the Ice Bucket.
6. On the Placement screen, do as follows.
   a. Drag the pools into the Placed Samples area on the right.
   b. Scan or type the barcode of the library tube strip into the container name field.
   c. Select Record Details.
   After exiting the Placement screen, the Validate Library Strip Tube Barcode automation makes sure that the library tube strip barcode conforms to the barcode mask. For the Library 8-tube Strip, the barcode mask is LC[0-9]{7}-L[A-Z]{1}1 and an error message displays if the barcode does not match. For the Library 2-tube Strip, the barcode mask is LC[0-9]{7}-L[A-Z]{1}2.
7. The fields displayed on the Record Details screen are used to create the planned run and generate the sample sheet.
   The analysis related information for the planned run is from the ACT associated with the samples and no further analysis configuration is required. Refer to the following table for details.

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Table 12 Fields Displayed on Record Details Screen of Load to Library Tube Strip (NovaSeq X Series Sequencing v1.1) Step

Field	Value
Run Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. No spaces.
Run Mode	The selected Run Mode must correspond to the ACT type. Presets • Local[1] • Cloud[2]
Read 1 Cycles	Presets • 151 • 101 • 51 • Type a custom value
Read 2 Cycles	Presets • 151 • 101 • 51 • Type a custom value
Index 1 Cycles	Presets • 0 • 6 • 8 • Type a custom value[3]
Index 2 Cycles	Presets • 0 • 6 • 8 • Type a custom value[3]

[1] The Local run mode generates the sample sheet. The Run/Analysis configuration is imported manually to the NovaSeq X Series instrument through the sample sheet. The sample sheet is downloaded and imported manually by the user on NovaSeq X Control Software to start the run. The downstream secondary analysis is done using the onboard DRAGEN module.
[2] The Cloud run mode sends the Run/Analysis configuration to ICA. This configuration is downloaded to the NovaSeq X Series instrument through ICA to start the run. The downstream secondary analysis is done in the cloud.
[3] The custom value must correspond to the longest index sequence of the samples in the pools in the library tube strip. Otherwise, the planned run creation fails and an error message displays.

8. Select Validate Run Setup and Create Planned Run to trigger the automation script. The script performs the following actions:
   - Validates the parameters entered on the Record Details screen.

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- Creates the planned run and sends it to ICA when the Run Mode is Cloud.
- Generates the sample sheet and attaches it to the placeholder in the Files area on the Record Details screen for all Run Mode types.

9. Select Next Steps.
   On the Assign Next Steps screen, the next step for the pooled samples is set to the AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.1) step.
10. Select Finish Step to advance the pooled samples to the AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.1) step. For more information on how to start the sequencing run for different run modes, refer to *NovaSeq X Series Integration v1.2 Configuration and User Interaction on page 1*.

Step 5: AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.1)

This protocol contains the AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.1) step. This step is fully automated.

Do not add samples to the Ice Bucket or start or complete the AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.1) step. The integration does this automatically.

The integration starts the step automatically and data from the run is parsed back into Clarity LIMS. User interaction is not required, but you can review the stages of the step in Clarity LIMS.

Review Run Data

Read summary metrics are recorded for the library pool. After the run is complete, open the step and review these metrics in the Step Details section and the Sample Details table.

Step Details Section

The following values populate the master step fields:

- Run Name
- Run Status
- Output Folder
- Current Read
- Current Cycle
- Library Tube Barcode
- Flow Cell ID
- Flow Cell Side
- Flow Cell Type
- Flow Cell Part Number

- Flow Cell Lot Number
- Flow Cell Expiration Date
- Instrument ID
- Instrument Type
- Instrument Control Software Version
- Sequencing Log

Sample Details Table

Summary metrics populate the following global custom fields:

- % Bases >=Q30 R1
- % Bases >=Q30 R2
- % Error Rate R1
- % Error Rate R2
- Yield (Gb) R1
- Yield (Gb) R2
- Reads PF R1
- Reads PF R2
- %PF R1
- %PF R2
- % Aligned R1
- % Aligned R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- Cluster Density R1
- Cluster Density R2

Step 6: AUTOMATED - Analysis Run (NovaSeq X Series Sequencing v1.1)

This protocol contains the AUTOMATED - Analysis Run (NovaSeq X Series Sequencing v1.1) step. This step is fully automated.

Do not add samples to the Ice Bucket or start or complete the AUTOMATED - Analysis Run (NovaSeq X Series Sequencing v1.1) step. The integration does this automatically.

The integration starts the step automatically and data from the run is parsed back into Clarity LIMS. User interaction is not required, but you can review the stages of the step in Clarity LIMS. If the run analysis is successful, the integration completes the step automatically.

Review Analysis Data

After the analysis run is complete, open the step and review the following values in the Step Details section:

- Analysis Status
- Analysis Result Location
- Log

After the analysis is complete, download the demultiplexing results and analysis results summary files from the file placeholders. Retrieve detailed analysis results from ICA.

The demultiplexing results file contains the following demultiplexing statistics for each sample:

- Number of reads
- Number of perfect index reads
- Number of mismatch index reads

The analysis result summary file provides a summary of what analysis workflows were performed. This file also identifies the total number of samples analyzed and how many were completed or failed for each analysis.

At this point, the entire NovaSeq X Series Integration workflow is fully validated.

Troubleshooting

If an automation trigger does not appear to run its corresponding scripts, refer to the *Troubleshooting Automation* section in the Clarity LIMS API & Database documentation.

Automated Step Does Not Start

If the AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.1) step does not start, Clarity LIMS is likely not receiving sequencing run events from BaseSpace Sequence Hub correctly. Check for the following issues:

1. Make sure that the NovaSeq X Control Software is configured properly as follows.
    a. Before you start the run, in the NovaSeq X Control Software, select the appropriate BaseSpace Sequence Hub region in the Hosting location drop-down list.
    b. Under Run Settings, check the boxes next to Cloud run monitoring and Cloud run storage.

If there are issues after using the configuration settings on the NovaSeq X Control Software, contact Illumina Support.

Automated Step Starts, But Does Not Complete

If the AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.1) step starts, but does not complete, do as follows.

1. Log in to the default user account and use one of the following methods to open the in progress step in Clarity LIMS:
   a. In Lab View, find the step in the Recent Activities pane.
   b. Search for the step in Clarity LIMS using the library tube strip barcode as the search term.
2. On the Record Details screen, the Sequencing Log multiline text field contains logging information. If unable to reach the Record Details screen, or if the Sequencing Log field does not contain enough information to resolve the issue, contact Illumina Support. Provide the relevant information from the troubleshooting steps already performed.

Error When Creating Planned Runs

If you receive an error when creating a planned run, check the log message in the Load to Library Tube Strip step to identify the issue. If you cannot correct the issue, contact Illumina Support.

Incompatible Analyses in a Planned Run

Only compatible analysis versions should be combined in a single planned run. When incompatible analysis versions are combined, an error log message displays. To resolve this error, check the ACTs that were used for the run and only select the ACTs that are compatible with the planned run.

Validate Analysis Configuration Automation Check Fails

The Validate Analysis Configuration automation check is in the Make Bulk Pool (upon pooling) and Load to Library Tube Strip (upon step entry) steps. If a failure happens, an error message displays and the step can be aborted, or you might be prevented from continuing the step. For NovaSeq X Series Integration v1.2, the configuration limits for the Cloud and Local modes are as follows.

- Cloud—Maximum of seven applications and genome configurations (and, if needed, the BCL Convert Only application). Cloud mode also allows up to eight different library prep kits for each application and genome configuration.
- Local—Maximum of three applications and genome configurations (and, if needed, the BCL Convert Only application). Local mode also allows up to eight different library prep kits for each application and genome configuration.

This check can fail for the following reasons:

- The analysis configuration after library pooling or during the planned run creation exceeds the configuration limit. Resolve this error as follows.

  - If the error occurs on the Make Bulk Pool step, reduce the number of analysis configurations for a pool. To reduce the number, reorganize the samples for the pooling step (eg, by pooling samples that have similar configurations together).
  - If the error occurs on the Load to Library Tube step, reduce the number of analysis configurations for the planned run by reorganizing the samples for multiple runs instead of a single run.
- ACTs of samples in the same pool or planned run must have the same run mode (Local or Cloud). Resolve this error as follows.
  1. Abort the step and remove samples with ACTs that have conflicting run modes from the Ice Bucket.
  2. Make sure that all remaining samples in the Ice Bucket have ACTs with the same run modes.
  3. Select Begin Work to continue the step.
- ACTs of samples in the same pool or planned run that have the same secondary analysis must have the same analysis version. Resolve this error as follows.
  - If the error occurs on the Make Bulk Pool step, pool the samples again. Make sure that samples with the same secondary analysis, but conflicting analysis versions, are in different pools.
  - If the error occurs on the Load to Library Tube step, reorganize the samples for the planned run. Make sure that samples with the same secondary analysis, but conflicting analysis versions, are in different runs.
- ACTs of samples in the same pool or planned run that have the same secondary analysis must have the same analysis settings. The analysis setting fields can differ for a different secondary analysis and are configured in BaseSpace Sequence Hub when the ACT is created. Resolve this error as follows.
  1. If the error occurs on the Make Bulk Pool step, pool the samples again. Make sure that samples with the same secondary analysis, but conflicting analysis settings, are in different pools.
  2. If the error occurs on the Load to Library Tube step, reorganize the samples for the planned run. Make sure that samples with the same secondary analysis, but conflicting analysis settings, are in different runs.

NovaSeq X Series Integration v1.2 Manual Upgrade

NovaSeq X Series Integration Package v1.2 includes modifications to the existing NovaSeq X Series Sequencing workflows to support the following new features:

- PhiX control included in the Dilute and Denature step instead of the Make Bulk Pool step.
- Support for the new 1.5B and 25B flow cells.
- Analysis tracking for the status and high level analysis result summary through the AUTOMATED - Analysis Run step (this is done at the metaworkflow level).

> Analysis tracking is a new step in NovaSeq X Series protocol and cannot be added to a non-pending protocol or workflow. For analysis tracking at the metaworkflow level, do not proceed with the manual upgrade. Instead, install the NovaSeq X Series v1.2 Integration Package with the NovaSeq X Series Sequencing v1.1 protocol.

- The following workflow updates are included in NovaSeq X Series Integration Package v1.2:
  - AUTOMATED - Sequencing Run step: Renamed the Library Tube Barcode master step custom field. This field is now named Library Tube Strip Barcode.
  - Load to Library Tube Strip step: Removed the Output Folder master step custom field.
  - Make Bulk Pool master step: Updated the dropdown items in the Final Loading Concentration (pM) custom field.

Use the following instructions to manually update the workflows.

Prerequisites

Illumina recommends upgrading to Illumina Preset Protocols (IPP) v2.7 to support the new features. If you do not have IPP v2.7 installed, do the following before starting the manual upgrade:

- Install `ClarityLIMS-NGS-Package-v5 RPM` v5.24.
- Replace the following files in `/opt/gls/clarity/extensions/conf/driverfiletemplates`:
  - `NovaSeqXSeries_Bulk_Pool1.csv`
  - `NovaSeqXSeries_Bulk_Pool2.csv`
  - `NovaSeqXSeries_Dilute_Denature_Calculate_Volumes.csv`

For copies of the replacement files, contact Illumina Support.

Add New Containers

1. On the Configuration tab, select Consumables, and then select Containers.
2. Add the following information to enable the Library 2-tube Strip container that supports the 1.5B flow cell:

   > No action is required for the 25B flow cell. The flow cell uses the same Library 8-tube Strip as the 10B flow cell.

   a. For Container Name, enter Library 2-tube Strip.
   b. For Rows, update the following fields:
      - Number: 2
      - Naming: Alphabetic
   c. For Columns, update the following fields:
      - Number: 1

- Naming: Numeric
- Start at: 1

Add New Master Step Fields

Enable PhiX control in the Dilute and Denature step as follows.

1. On the Configuration tab, select Custom Fields, and then select Master Step Fields.
2. Add the following master step fields for the Dilute and Denature (NovaSeq X Series Sequencing v1.x) step:

Table 13 Dilute and Denature (NovaSeq X Series Sequencing v1.x) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
PhiX Volume (µl)	Numeric	Read Only	Decimal places displayed: 1
1–2% PhiX Spike-In	Toggle Switch	Required Field	Default • No

Modify Existing Master Step Fields

1. On the Configuration tab, select Custom Fields, and then select Master Step Fields.
2. Modify the following master step fields:

Table 14 Master Step Field Modifications

Master Step	Field	Modification	Purpose/Notes
Make Bulk Pool (NovaSeq X Series Sequencing v1.x)	Final Loading Concentration (pM)	1. Update Dropdown Items with the following options: • 90 • 140 • 150 • 160 • 180	• Only perform this modification if the new Dropdown Items are required. • This modification is not required to enable support for the new flow cells or PhiX control in the Dilute and Denature (NovaSeq X Series Sequencing v1.x) step.
	Flowcell Type	1. Change Field Type from Text to Text Dropdown. 2. Update Field Options as follows. • Read Only: = No • Custom Entries = No 3. Update Dropdown Items with the following options: • 1.5B • 10B • 25B	This modification is required to support the new flow cells.

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Master Step	Field	Modification	Purpose/Notes
Load to Library Tube Strip (NovaSeq X Series Sequencing v1.x)	Output Folder	1. Remove the field.	• If the step has been run previously and this field is used, it cannot be removed. This field does not affect the functionality of the workflow. • This modification is not required to enable support for the new flow cells or PhiX control in the Dilute and Denature (NovaSeq X Series Sequencing v1.x) step.

Master Step	Field	Modification	Purpose/Notes
AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.x)	Library Tube Barcode	1. Rename the field from Library Tube Barcode to Library Tube Strip Barcode.	• If the installed NovaSeq X Series workflow is v1.1 or earlier, this modification is not required to enable support for the new flow cells or PhiX control in the Dilute and Denature (NovaSeq X Series Sequencing v1.x) step. • For NovaSeq X Series workflow v1.2, this modification is required. Run event handling fails without this modification.

Modify Existing Global Field

1. On the Configuration tab, select Custom Fields, and then select Global Fields.
2. For the NovaSeq X Flowcell Type field, update the Dropdown Items with the following values:

   A merge conflict is expected when upgrading from IPP v2.6 to v2.7 due to the changes in the NovaSeq X Flowcell Type field.

   - 1.5B
   - 10B
   - 25B

Add, Configure, and Modify Automations

Add Automation

1. On the Configuration tab, select Automation, and then select Step Automation.

2. On the Load to Library Tube Strip (NovaSeq X Series Sequencing v1.x) step, add the following automation to enable new flow cell types:
   - Name—Validate Flowcell Inputs and Validate Analysis Configurations and Register Step Started
   - Channel Name—limsserver
   - Command line:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} \
script:validate_same_udf_value_for_analytes -f 'Run Mode' -f 'NovaSeq X Flowcell
Type' \
script:validate_selected_container \
-fn 'NovaSeq X Flowcell Type' -fv '1.5B' -ct 'Library 2-tube Strip' \
-fn 'NovaSeq X Flowcell Type' -fv '10B' -ct 'Library 8-tube Strip' \
-fn 'NovaSeq X Flowcell Type' -fv '25B' -ct 'Library 8-tube Strip' && \
/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/novaseqxserie
s-automation.jar -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} -m PerRun script:validate_analysis_config
script:validate_physical_logical_configurations && \
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s
'com/illumina/upa/scripts/common/step_started.groovy'"
```

Configure Automation Settings

1. On the Configuration tab, select Lab Work.
2. Configure the trigger location and style for the following automations:

Table 15 Automation Configuration

Step	Automation	Setting	Purpose/Notes
Load to Library Tube Strip (NovaSeq X Series Sequencing v1.x)	Validate Flowcell Inputs and Validate Analysis Configurations and Register Step Started	• Trigger Location: Step • Trigger Style: Automatic upon entry	These configuration updates are required to enable the new flow cell types.
	Validate Input Count and Validate Analysis Configurations and Register Step Started	Trigger Location: Not used	

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Step	Automation	Setting	Purpose/Notes
Dilute and Denature (NovaSeq X Series Sequencing v1.x)	Calculate Volumes	• Trigger Location: Record Details • Trigger Style: Manual button	This configuration update is required to enable PhiX control on the Make Bulk Pool (NovaSeq X Series Sequencing v1.x) step.

Modify Step Automations

1. On the Configuration tab, select Automation, and then select Step Automation.
2. Select the Calculate Volumes automation that is enabled on the Make Bulk Pool (NovaSeq X Series Sequencing v1.x) step.
3. Update the existing command line as follows.
   This modification is required to move the PhiX control from the Make Bulk Pool (NovaSeq X Series Sequencing v1.x) step to the Dilute and Denature (NovaSeq X Series Sequencing v1.x) step. Changes are highlighted in red.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} -
log {compoundOutputFileLuid1} \
script:evaluateDynamicExpression \
-exp 'if (!input.hasValue(::Molarity (nM)::)) { return; }; if (output.hasValue
(::Number of Samples in Pool::)) { output.::Number of Samples in Pool:: =
output.::Number of Samples in Pool:: + 1; } else { output.::Number of Samples in
Pool:: = 1; }' -t true \
script:evaluateDynamicExpression \
-exp 'output.::Bulk Pool Volume (ul):: = 100 * step.::Number of Lanes to
Sequence::;
output.::NovaSeq X Flowcell Type:: = step.::Flowcell Type::;
output.::Final Loading Concentration (pM):: = step.::Final Loading Concentration
(pM)::;
input.::Per Sample Volume (ul):: = 2 * output.::Bulk Pool Volume (ul):: /
input.::Molarity (nM):: / output.::Number of Samples in Pool::;
output.::Total Sample Volume (ul):: = 0;' -t true \
script:calculate_multipool_adjusted_per_sample_volume -t \
script:evaluateDynamicExpression \
```


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```
-exp 'if (output.hasValue(::Total Sample Volume (ul)::)) { output.::Total Sample
Volume (ul):: = output.::Total Sample Volume (ul):: + input.::Adjusted Per
Sample Volume (ul)::; } else { output.::Total Sample Volume (ul):: =
input.::Adjusted Per Sample Volume (ul)::; }' -t true \
script:evaluateDynamicExpression \
-exp 'if (output.::Total Sample Volume (ul):: > output.::Bulk Pool Volume
(ul)::) { output.::RSB Volume (ul):: = 0 } else { output.::RSB Volume (ul):: =
output.::Bulk Pool Volume (ul):: - output.::Total Sample Volume (ul):: }' -t
true \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeqXSeries_Bulk_
Pool1.csv -o 1.csv \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeqXSeries_Bulk_
Pool2.csv -o 2.csv \
&& cat 1.csv 2.csv > {compoundOutputFileLuid0}.csv \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} -
log {compoundOutputFileLuid1} \
script:evaluateDynamicExpression \
-exp 'output.::Number of Samples in Pool:: = ::::; output.::Total Sample Volume
(ul):: = ::::; output.::Bulk Pool Volume (ul):: = ::::;' -t true \
&& echo 'Calculate Volume completed successfully'"
```

4. Select the Calculate Volumes automation that is enabled on the Dilute and Denature (NovaSeq X Series Sequencing v1.x) step.
5. Update the existing command line as follows.
   This modification is required to move the PhiX control from the Make Bulk Pool (NovaSeq X Series Sequencing v1.x) step to the Dilute and Denature (NovaSeq X Series Sequencing v1.x) step and to enable support for the 1.5B and 25B flow cells. Changes are highlighted in red.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} -
log {compoundOutputFileLuid1} \
script:evaluateDynamicExpression \
-exp '
if (input.::NovaSeq X Flowcell Type:: == ::10B:: || input.::NovaSeq X Flowcell
Type:: == ::1.5B::) {
output.::NaOH Volume (ul):: = 8.5;
output.::TT2 Volume (ul):: = 127.5;
```


For Research Use Only. Not for use in diagnostic procedures.

```
output.::BP Aliquot Volume (ul):: = input.::Final Loading Concentration (pM):: *
170 / (2 * 1000);
output.::RSB Volume (ul):: = 34 - output.::BP Aliquot Volume (ul)::;
if (step.::1-2% PhiX Spike-In::) { output.::PhiX Volume (ul):: = 1;
output.::PhiX Concentration (pM):: = 300; }
else { output.::PhiX Volume (ul):: = ::::; output.::PhiX Concentration (pM):: =
::::; };
} else {
output.::NaOH Volume (ul):: = 14;
output.::TT2 Volume (ul):: = 210;
output.::BP Aliquot Volume (ul):: = input.::Final Loading Concentration (pM):: *
280 / (2 * 1000);
output.::RSB Volume (ul):: = 56 - output.::BP Aliquot Volume (ul)::;
if (step.::1-2% PhiX Spike-In::) { output.::PhiX Volume (ul):: = 1.6;
output.::PhiX Concentration (pM):: = 300; }
else { output.::PhiX Volume (ul):: = ::::; output.::PhiX Concentration (pM):: =
::::; };
}' && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeqXSeries_Dilute_
Denature_Calculate_Volumes.csv -o {compoundOutputFileLuid0}.csv -q true -
destLIMSID {compoundOutputFileLuid0} -l {compoundOutputFileLuid1} \
&& echo; echo 'Calculate Volumes completed successfully.'"
```

6. Select the Set Next Step automation that is enabled on the Dilute and Denature (NovaSeq X Series Sequencing v1.x) step.
7. Update the existing command line as follows.
   This modification is required to enable support for the 1.5B and 25B flow cells and future 8-tube library strips. Changes are highlighted in red.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
script:evaluateDynamicExpression -t false -h false -exp 'output.::Run Mode:: =
input.::Run Mode::; output.::NovaSeq X Flowcell Type:: = input.::NovaSeq X
Flowcell Type::; nextStep = ::ADVANCE::' -log {compoundOutputFileLuid1}"
```

8. Select the Validate Library Tube Strip Barcode automation that is enabled on the Load to Library Tube Strip Barcode (NovaSeq X Series Sequencing v1.x) step.
9. Update the existing command line as follows.
   This modification is required to enable support for the 1.5B and 25B flow cells and future 8-tube library strips. Changes are highlighted in red.

For Research Use Only. Not for use in diagnostic procedures.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
script:validate_output_containers -l {compoundOutputFileLuid1} -r 'Library 8-
tube Strip:LC[0-9]{7}-L[A-Z]{1}1' -r 'Library 2-tube Strip:LC[0-9]{7}-L[A-Z]
{1}2' -max 1"
```

Modify Existing Steps

Enable PhiX Control

1. On the Configuration tab, select Lab Work.
2. Select the Make Bulk Pool (NovaSeq X Series Sequencing v1.x) step.
3. For Control Types, remove PhiX v3.
4. For the Record Details milestone, navigate to Step Data and remove the following fields:
   - Final Loading Volume (µl)
   - % PhiX (2.0 nM) Spike-In
5. Select the Dilute and Denature (NovaSeq X Series v1.x) step.
6. Select the Record Details milestone.
7. Navigate to Step Data and add 1-2% PhiX Spike-In to Master Step Fields.

Enable New Flow Cell Types

1. On the Configuration tab, select Lab Work.
2. Select the Load to Library Tube Strip (NovaSeq X Series Sequencing v1.x) step.
3. Select the Queue milestone.
4. Navigate to Sample Table and add the NovaSeqX Flowcell Type field to Column Headers.
5. Select the Ice Bucket milestone.
6. Navigate to Sample Table and add the NovaSeqX Flowcell Type field to Column Headers.
7. Select the Placement milestone.
8. Navigate to Destination Containers and add Library 2-tube Strip.

NovaSeq X Series Integration v1.2 Release Notes

These Release Notes describe the key changes to software components for the Clarity LIMS NovaSeq X Series Integration Package v1.2.

Compatibility

This version of the integration package is compatible with the following software:

- Clarity LIMS v6.2 and later (with a BaseSpace Sequence Hub account that has the Enterprise or Professional subscription)
- NGS Extensions Package v5.24 and later

- Illumina Preset Protocols (IPP) v2.7.0 and later
- NovaSeq X Series Control Software v1.2.0

New Features

- Supports analysis tracking at the metaworkflow level for analysis status and high level analysis summary results.
- Supports the new 1.5B and 25B flow cell types. The B3 flow cell has been renamed to the 10B flow cell.
- Uses the SecureString type for improving the security of values stored in the AWS param store.
- Includes the following improvements to the NovaSeq X Series Sequencing v1.1 protocol:
  - Removes the unused Output Folder step custom field from the Load to Library Tube Strip step.
  - Renames the Library Tube barcode step custom field to Library Tube Strip Barcode in the AUTOMATED - Sequencing Run step.

Defects Fixed

- None

Known Limitations

- You must log in to BaseSpace Sequence Hub to retrieve the cloud analysis results. For local analysis, the secondary analysis results are located in the external storage configured in Illumina Run Manager. The analysis step tracks only the first run, but not subsequent queued runs.
- The Local run mode does not support custom library prep and index adapter kits.

Known Issues

- On the Make Bulk Pool step, the log displays a warning when the Calculate Volume automation is triggered and at least one pool consists of multiple inputs. This issue is caused by the output custom field being reset multiple times at the end of the automation. This issue does not affect the volume calculation.
- On the Dilute and Denature step, the BP Aliquot Volume value is rounded up to the nearest µl. The value in the calculation file is reflected with up to two decimal places.
- All leading and trailing spaces are removed from the ACT names and ACTs created in BaseSpace Sequence Hub. Names containing leading or trailing spaces may not be recognized by Clarity LIMS. Make sure that ACTs created in BaseSpace Sequence Hub do not have leading or trailing spaces.

NovaSeq X Series Integration v1.1 Configuration and User Interaction

The Illumina NovaSeq X Series Integration Package v1.1 supports the integration of Clarity LIMS to Illumina NovaSeq X series instruments.

For instructions on validating and troubleshooting the Illumina NovaSeq X Series Integration, refer to *NovaSeq X Series Integration v1.1 Validation and Troubleshooting* on page 86.

The configuration provided in this integration has been established to support NovaSeq X Series lab processes. Any configuration changes to protocols or workflows (including renaming protocols, steps, and fields) could break the process.

Overview

The integration includes the following features:

- Preconfigured NovaSeq X Series Sequencing v1.0 workflow that maps to lab protocols and instrument runs.
- The following steps in the NovaSeq X Series Sequencing v1.0 preconfigured protocol:
  - Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.0)
  - Make Bulk Pool (NovaSeq X Series Sequencing v1.0)
  - Dilute and Denature (NovaSeq X Series Sequencing v1.0)
  - Load to Library Tube Strip (NovaSeq X Series Sequencing v1.0)
  - AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0)
- Automatic validation of run setup information before sample sheet generation or planned run creation.
- Automated generation of the sample sheet. This sample sheet is used to start the sequencing run on the NovaSeq X Series Control Software through Local run mode.
- Automated creation of a planned run on Illumina Connected Analytics (ICA). The NovaSeq X Series Control Software retrieves the planned run for use with the Cloud run mode.
- Automated tracking of the NovaSeq X Series sequencing run and parsing of run statistics into Clarity LIMS.
- Support for configuration of multiple secondary analyses with multiple library prep kits (LPKs) and index adapter kits (IAKs) for the planned run. The analysis is either performed by ICA in Cloud mode or by DRAGEN on-board the instrument in Local mode.
- [Optional] Preconfigured Library Prep Validation v2.3.1 workflow used for validation purposes only. The workflow contains a single-step protocol that models the library prep required to produce libraries that are ready for the NovaSeq X Series Sequencing v1.0 workflow. For more information, refer to *NovaSeq X Series Integration v1.1 Validation and Troubleshooting* on page 86.
- [Optional] Clarity LIMS Product Analytics (CLPA) integration that can be used with ICA. The CLPA integration installation and configuration is required. For more information, refer to *Clarity LIMS Product Analytics Integration v1.2 Configuration and User Interaction* on page 2399.

Prerequisites and Assumptions

Analysis Configuration Template

The NovaSeq X Series Sequencing v1.0 workflow uses analysis configuration templates (ACTs) to configure secondary analysis for planned runs. Required ACTs must be created using BaseSpace Sequence Hub. The ACT names must be unique. The index adapter kit (the label group in Clarity LIMS) selected in the ACT is created in Clarity LIMS. The same label group must be used in the library preparation step. For more information, refer to *NovaSeq X Series Integration v1.1 Validation and Troubleshooting* on page 86.

Make sure that ACT names created in BaseSpace Sequence Hub do not have leading or trailing spaces. Otherwise, Clarity LIMS can have issues with recognizing the ACT names.

Samples

Samples must go through the library preparation and quantification process before they enter the NovaSeq X Series Sequencing v1.0 workflow. It is assumed that the following steps have completed before samples are assigned to the workflow:

- Samples have been accessioned into Clarity LIMS.
- Samples have been run through QC and library prep.
- Samples have the Molarity (nM) global field set to a value. This value is required for the Calculate Volumes automation in the Make Bulk Pool step.

For more information on sample accessioning, refer to *Sample Accessioning* and *Upload and Modify Samples* in the Getting Started section of the Clarity LIMS Core Functionality documentation.

You can assign samples to workflows automatically, using a routing script, or manually—from the Projects & Samples dashboard. Refer to *Assign and Process Samples* in the Clarity LIMS Core Functionality documentation.

Workflows, Protocols, and Steps

The NovaSeq X Series Integration Package v1.1 includes the following workflows:

- NovaSeq X Series Sequencing v1.0
- Library Prep Validation v2.3.1 (optional, but recommended for validation purposes)

The following table describes the protocols and steps included in these workflows.

Table 16 NovaSeq X Series Integration Package v1.1 Protocols and Steps

Protocol Name	Protocol Purpose	Protocol Steps
Library Prep Validation v2.3.1 Workflow		

Protocol Name	Protocol Purpose	Protocol Steps
Library Prep Validation v2.3.1	Included for validation purposes only, this protocol models the library prep steps required to advance samples to the NovaSeq X Series Sequencing v1.0 workflow. The protocol contains a single step - Library Prep Validation v2.3.1. After this step, a routing script sends the samples to the first step of the NovaSeq X Series Sequencing v1.0 workflow (Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.0)).	1. Library Prep Validation v2.3.1

NovaSeq X Series Sequencing v1.0 Workflow

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Protocol Name	Protocol Purpose	Protocol Steps
NovaSeq X Series Sequencing v1.0	This protocol models the lab processes of run setup for starting a NovaSeq X Series sequencing run.	1. Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.0) • The secondary analysis of samples is configured using the ACT. • The labels applied to the samples are validated against the selected ACT so that samples use valid labels. 2. Make Bulk Pool (NovaSeq X Series Sequencing v1.0) • The Calculate Volumes script assists with pooling samples and adding resuspension buffers and reagents. • The Validate Analysis Configurations automation validates the ACT of pooled samples to make sure that the secondary analysis configurations remain valid after pooling. 3. Dilute and Denature (NovaSeq

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Validation Workflow

The Library Prep Validation v2.3.1 workflow allows for validation of the system after installation is complete. For details, refer to *NovaSeq X Series Integration v1.1 Validation and Troubleshooting* on page 86.

Step Automations

The following table lists the step automations included in the NovaSeq X Series Sequencing v1.0 workflow. Automation details are provided in the documentation for each step.

For details on the validation workflow, refer to *NovaSeq X Series Integration v1.1 Validation and Troubleshooting* on page 86.

Table 17 NovaSeq X Series Sequencing v1.0 Workflow Step Automations

Protocol	Step	Automation
NovaSeq X Series Sequencing v1.0	1. Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.0)	Register Step Completed[1] Retrieve ACT Information Validate Reagent Labels and Apply Selected ACT to Samples and Set Next Step Validate Sample Names and Retrieve Analysis Configuration Template List and Register Step Started[2]
	2. Make Bulk Pool (NovaSeq X Series Sequencing v1.0)	Calculate Volumes Register Pools and Register Step Completed[1] Register Step Started[1] Set Next Step Validate Analysis Configurations
	3. Dilute and Denature (NovaSeq X Series Sequencing v1.0)	Calculate Volumes Register Step Completed[1] Set Next Step Validate Inputs Flowcell Type and Register Step Started[2]
	4. Load to Library Tube Strip (NovaSeq X Series Sequencing v1.0)	Register Step Completed[1] Set Next Step Validate Analysis Configurations and Register Step Started[2] Validate Library Tube Strip Barcode Validate Run Setup and Create Planned Run
	5. AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0)	Register NovaSeq X RunCompleted and Register Step Completed[1] Register Step Started and Register NovaSeq X Run and Register NovaSeq X Run Association and Register NovaSeq X RunStarted[1]

[1] These automations are required for CLPA support only.

[2] These automations are required for the NovaSeq X Series Sequencing v1.0 workflows and contain additional logic needed for CLPA support. If you would like to remove support for CLPA, contact Illumina Support.

All remaining automations are required for the NovaSeq X Series Sequencing v1.0 workflows.

The Illumina Universal Sample Identifier is a global field that is imported with the Library Prep Validation v2.3.1 and NovaSeq X Series Sequencing v1.0 workflows. Illumina Universal Sample Identifier is a text field that is reserved for CLPA support. The field is optional and not required for this integration.

Step 1: Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.0)

The Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.0) step uses ACTs to configure secondary analysis for samples. Each ACT contains details related to the secondary analysis (eg, index adapter kit to use, reference genome setting, and so on). You can select an ACT from a list and assign samples to it. A planned run can have multiple ACTs and this step must be repeated for each ACT that is required for the run. For more information on creating ACTs and assigning samples to them, refer to *NovaSeq X Series Integration v1.1 Validation and Troubleshooting* on page 86.

The following automations are configured on this step:

- Validate Sample Names and Retrieve Analysis Configuration Template List and Register Step Started
- Retrieve ACT Information
- Validate Reagent Labels and Apply Selected ACT to Samples and Set Next Step
- Register Step Completed

Validate Sample Names and Retrieve Analysis Configuration Template List and Register Step Started Automation

Automatically triggered on entry to the step, this automation completes the following actions:

- Checks that submitted sample names contain only alphanumeric, dash, and underscore characters.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
script:evaluateDynamicExpression -exp 'submittedSamples.each { sample -> if (
!sample.name.matches( ::[A-Za-z0-9_-]+:: ) ) { fail ( ::Submitted sample :: +
sample.name + :: should only have alphanumeric, dash, and underscore characters.
Please rename and try again.:: ) } }' -log {compoundOutputFileLuid1}
```

- Retrieves the list of ACTs that were created in BaseSpace Sequence Hub.

```
/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/novaseqxserie
s-automation.jar script:retrieve_act_list -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1}
```

- Registers the start time of the step by publishing messages to CLPA through the ICA.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s
'com/illumina/upa/scripts/common/step_started.groovy'
```

Retrieve ACT Information Automation

Automatically triggered when you select Retrieve ACT Information on the Record Details screen, this automation completes the following actions:

- Retrieves the details of the selected ACT and populates the step custom fields (eg, Analysis Version, Library Prep Kit, and so on).
- Saves the details into a file that is available to download. This file is stored in the Analysis Configuration Metadata file placeholder.

```
/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/novaseqxserie
s-automation.jar script:retrieve_act_info -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} -a '{udf:Analysis Configuration
Template}'
```

Validate Reagent Labels and Apply Selected ACT to Samples and Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation completes the following actions:

- Checks that the molarity of all samples is specified.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
script:evaluateDynamicExpression -t false -h false -exp 'if (!input.hasValue
(::Molarity (nM)::)) { fail(::The Molarity cannot be empty.::) ; } else if
(input.::Molarity (nM):: <= 0) { fail(::The Molarity cannot be negative or
zero.::) ; }' -log {compoundOutputFileLuid1}
```

- Checks that all samples are indexed and for invalid indexes. The indexes applied are the same as the selected ACT.

```
/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/novaseqxserie
s-automation.jar script:validate_indexes -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} -a '{udf:Analysis Configuration
Template}'
```

- Assigns the selected ACT to all samples in the step.

```
/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/novaseqxseries-automation.jar script:assign_act -i {stepURI:v2} -u {username} -p {password} -a '{udf:Analysis Configuration Template}' -l {compoundOutputFileLuid1}
```

- Sets the next step for samples to ADVANCE, which advances them to the next step in the protocol (Make Bulk Pool (NovaSeq X Series Sequencing v1.0)).

```
nextStep = ::ADVANCE:
```

Register Step Completed Automation

Automatically triggered on exit of the step, this automation registers completion time of the step by publishing messages to CLPA through ICA.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s 'com/illumina/upa/scripts/common/step_completed.groovy'
```

Master Step Fields

The following table lists the configuration details for fields that are defined on the Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.0) step.

Table 18 Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.0) Master Step Field Configuration

Field Name	Field Type	Options
Analysis Configuration Template	Text Dropdown	Required Field
Application	Text	Read Only
Application Version	Text	Read Only
Index Adapter Kit	Text	Read Only
Library Prep Kit	Text	Read Only
Reference Genome	Text	Read Only
Secondary Analysis Mode	Text	Read Only

Global Fields

The following table lists the global custom fields that are configured to display on the Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.0) step.

Table 19 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
Molarity (nM)	Numeric		Decimal places displayed = 2
ACT Name	Text	Read Only	

User Interaction

For more information, refer to the Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.0) step section in *NovaSeq X Series Integration v1.1 Validation and Troubleshooting* on page 86.

Step 2: Make Bulk Pool (NovaSeq X Series Sequencing v1.0)

Samples are pooled in the Make Bulk Pool (NovaSeq X Series Sequencing v1.0) step. You can manually create working pools based on the final loading concentration required.

The following automations are configured on this step:

- Register Step Started
- Validate Analysis Configurations
- Calculate Volumes
- Set Next Step
- Register Pools & Register Step Completed

Register Step Started Automation

Automatically triggered on entry to the step, this automation registers the start time of the step by publishing messages to CLPA through ICA.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_started.groovy'
```

Validate Analysis Configurations Automation

Automatically triggered on exit of the Pooling screen, this automation performs analysis configuration validity and checks each pool for the following characteristics:

- Pooled samples are within the maximum configuration limit.
- Pooled samples have the same type of analysis (eg, Cloud or Local).
- Pooled samples that have the same secondary analysis also have the same analysis version (eg, v3.8.4) and analysis settings (eg, Map/Align Output Format = CRAM).

```
/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/novaseqxserie
s-automation.jar script:validate_physical_logical_configurations -i {stepURI:v2}
-u {username} -p {password} -l {compoundOutputFileLuid1} -m PerPool
script:validate_analysis_config -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} -m PerPool && /opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar
script:validate_same_analyte_udf_value_for_all_inputs -i {stepURI:v2} -u
{username} -p {password} -l {compoundOutputFileLuid1} -f 'Run Mode' -perPool
true
```

Calculate Volumes Automation

Automatically triggered when you select Calculate Volumes on the Record Details screen, this automation completes the following actions:

- Proceeds if the sample has molarity values.

```
if (!input.hasValue(::Molarity (nM)::)) { return; };
```

- Uses Number of Samples in Pool to calculate the volumes needed for the 2 nM intermediate library pools.

```
if (output.hasValue(::Number of Samples in Pool::)) { output.::Number of Samples
in Pool:: = output.::Number of Samples in Pool:: + 1; } else { output.::Number
of Samples in Pool:: = 1; }' -t true
```

- Uses the Bulk Pool Volume (µl) field and Number of Lanes to Sequence (x100) for the B3 flow cell type to calculate volumes needed for the 2 nM intermediate library pools.
- Copies the Final Loading Concentration (pM) and Flowcell Type from the step inputs to the step outputs.

```
if (step.::Flowcell Type:: == ::B3::) { output.::Bulk Pool Volume (ul):: = 100 *
step.::Number of Lanes to Sequence::; }; output.::NovaSeq X Flowcell Type:: =
step.::Flowcell Type::; output.::Final Loading Concentration (pM):: =
step.::Final Loading Concentration (pM)::;
```

- Calculates the per sample volume required for each library to make a 2 nM intermediate library pool.

```
input.::Per Sample Volume (ul):: = 2 * output.::Bulk Pool Volume (ul):: /
input.::Molarity (nM):: / output.::Number of Samples in Pool::;
```

- If PhiX is used for a planned run (eg, a value for % PhiX (2.0 nM) Spike-In is specified), sets the PhiX Volume (µl).

```
if (step.hasValue(::% PhiX (2.0nM) Spike-In::)) { step.::PhiX Volume (ul):: =
step.::% PhiX (2.0nM) Spike-In:: / 100 * step.::Final Loading Volume (ul)::; };
```

- Calculates the adjusted per sample volume for the pools.

```
script:calculate_multipool_adjusted_per_sample_volume -t
```

- Uses the Total Sample Volume (µl) to calculate the RSB volume (µl) required to top up the pools that are needed to create the 2 nM intermediate library pools. The RSB volume is saved to the file in the Calculation File placeholder in the Files section.

```
script:evaluateDynamicExpression \
-exp 'if (output.hasValue(::Total Sample Volume (ul)::)) { output.::Total Sample
Volume (ul):: = output.::Total Sample Volume (ul):: + input.::Adjusted Per
Sample Volume (ul)::; } else { output.::Total Sample Volume (ul):: =
input.::Adjusted Per Sample Volume (ul)::; }' -t true \
script:evaluateDynamicExpression \
-exp 'if (output.::Total Sample Volume (ul):: > output.::Bulk Pool Volume
(ul)::) { output.::RSB Volume (ul):: = 0 } else { output.::RSB Volume (ul):: =
output.::Bulk Pool Volume (ul):: - output.::Total Sample Volume (ul):: }' -t
true \
```

- Uses the `NovaSeqXSeries_Bulk_Pool1.csv` and `NovaSeqXSeries_Bulk_Pool2.csv` template files to generate a single CSV file. This file contains information about the pools and the samples that they contain. The generated file is stored in the Calculation File placeholder in the Files section.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeqXSeries_Bulk_
Pool1.csv -o 1.csv \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeqXSeries_Bulk_
Pool2.csv -o 2.csv \
&& cat 1.csv 2.csv > {compoundOutputFileLuid0}.csv \
```

- Resets the Number of Samples in Pool, Total Sample Volume (µl), and Bulk Pool Volume (µl) to null before exiting the step.

```
-exp 'output.::Number of Samples in Pool:: = ::::; output.::Total Sample Volume
(ul):: = ::::; output.::Bulk Pool Volume (ul):: = ::::;' -t true
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation completes the following actions:

- Proceeds only if the sample has a molarity value.
- Copies the Run Mode from input to output.
- Sets the next step for samples to ADVANCE, which advances them to the Dilute and Denature (NovaSeq X Series Sequencing v1.0) step.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} -
log {compoundOutputFileLuid1} \
script:evaluateDynamicExpression -exp 'if (!input.hasValue(::Molarity (nM)::)){
return; }; output.::Run Mode:: = input.::Run Mode::; nextStep = ::ADVANCE::;'"
```

Register Pools & Register Step Completed Automation

Automatically triggered on exit of the step, this automation completes the following actions:

- Registers the pool and library information used by the Clarity LIMS workflow. Samples in the pool are assumed to have gone through library prep.
- Registers completion time of the step by publishing messages to CLPA through ICA.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s
'com/illumina/upa/scripts/common/pooling.groovy' && /opt/gls/clarity/bin/java -
jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-
product-analytics-automation.jar script:executeUPAAutomationScript -i
{stepURI:v2} -u {username} -p {password} -s
'com/illumina/upa/scripts/common/step_completed.groovy'
```

Master Step Fields

The following table lists configuration details for the fields that are defined on the Make Bulk Pool (NovaSeq X Series Sequencing v1.0) step.

Table 20 Make Bulk Pool (NovaSeq X Series Sequencing v1.0) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
% PhiX (2.5 nM) Spike-In	Numeric		Range = 0–100

Field Name	Field Type	Options	Additional Options and Dropdown Items
Final Loading Concentration (pM)	Numeric Dropdown	Required Field Custom Entries	
Final Loading Volume (µl)	Numeric	Required Field	Default • 200
Flowcell Type	Text	Required Field Read Only	Default • B3
Minimum Per Sample Volume (µl)	Numeric	Required Field	Decimal places displayed = 2 Default • 2
Number of Lanes to Sequence	Numeric	Required Field	Range = 1–10 Decimal places displayed = 0
PhiX Volume (µl)	Numeric	Read Only	Hidden Decimal places displayed = 1 Used for Calculate Volume script when there is PhiX spike-in. Do not remove, rename, or modify this field as it can cause the integration to break.

Global Fields

The following table lists the global custom fields that are configured to display on the Make Bulk Pool (NovaSeq X Series Sequencing v1.0) step.

Table 21 Global Custom Fields Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			

Field Name	Field Type	Options	Additional Options and Dropdown Items
Final Loading Concentration (pM)	Numeric Dropdown	Required Field Custom Entries	Decimal places displayed = 0 Presets • 225 • 400
RSB Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
NovaSeq X Flowcell Type	Text	Required Field Read Only	Default • B3

User Interaction

For more information, refer to Run Make Bulk Pool (NovaSeq X Series Sequencing v1.0) step section in *NovaSeq X Series Integration v1.1 Validation and Troubleshooting* on page 86.

Step 3: Dilute and Denature (NovaSeq X Series Sequencing v1.0)

The Dilute and Denature (NovaSeq X Series Sequencing v1.0) step allows you to dilute pooled samples with the addition of RSB.

The following automations are configured on this step:

- Validate Inputs Flowcell Type and Register Step Started
- Calculate Volumes
- Set Next Step
- Register Step Completed

Validate Inputs Flowcell Type and Register Step Started

Automatically triggered on entry to the step, this automation completes the following actions:

- Checks that the specified flow cell type is valid.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
script:evaluateDynamicExpression -h false -exp 'if (!input.hasValue(::NovaSeq X
Flowcell Type::)){fail(::Invalid Flowcell Type custom field value. Please make
sure all input samples have a value assigned for Flowcell Type custom
field.::)}' -log {compoundOutputFileLuid1}
```

- Registers the start time of the step by publishing messages to CLPA through ICA.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s 'com/illumina/upa/scripts/common/step_started.groovy'
```

Calculate Volumes Automation

Automatically triggered when you enter the Record Details screen, this automation completes the following actions:

- Checks that the NovaSeq X Flowcell Type is B3 and sets NaOH Volume (µl) and TT2 Volume (µl) field values to 10 and 150.
- Computes BP Aliquot Volume (µl) and RSB Volume (µl) required for the dilution of pools to the required final loading concentration.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} -log {compoundOutputFileLuid1} \
script:evaluateDynamicExpression \
-exp 'if (input.::NovaSeq X Flowcell Type:: == ::B3::) { output.::NaOH Volume (ul):: = 10; output.::TT2 Volume (ul):: = 150; }; output.::BP Aliquot Volume (ul):: = input.::Final Loading Concentration (pM):: * 200 / (2 * 1000); output.::RSB Volume (ul):: = 40 - output.::BP Aliquot Volume (ul)::;'
```

- Generates a single CSV file that contains information about the reagents volume required to dilute the working pools. The generated file is stored in the Calculation File placeholder in the Files section.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p {password} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeqXSeries_Dilute_Denature_Calculate_Volumes.csv -o {compoundOutputFileLuid0}.csv -q true -destLIMSID {compoundOutputFileLuid0} -l {compoundOutputFileLuid1}"
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation completes the following actions:

- Copies the Run Mode from input to output.
- Sets the next step for samples to ADVANCE, advancing them to the Load to Library Tube Strip (NovaSeq X Series Sequencing v1.0) step.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
script:evaluateDynamicExpression -t false -h false -exp 'output.::Run Mode:: =
input.::Run Mode::; nextStep = ::ADVANCE::' -log {compoundOutputFileLuid1}
```

Register Step Completed Automation

Automatically triggered on exit of the step, this automation registers the completion time of the step by publishing messages to CLPA through ICA.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s
'com/illumina/upa/scripts/common/step_completed.groovy'
```

Global Fields

The following table lists the global custom fields that are configured to display on the Dilute and Denature (NovaSeq X Series Sequencing v1.0) step.

Table 22 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
BP Aliquot Volume (µl)	Numeric	Read Only	Decimal places displayed = 0
NaOH Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
RSB Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
TT2 Volume (µl)	Numeric	Read Only	Decimal places displayed = 2

User Interaction

For more information, refer to the Dilute and Denature (NovaSeq X Series Sequencing v1.0) step section in *NovaSeq X Series Integration v1.1 Validation and Troubleshooting* on page 86.

Step 4: Load to Library Tube Strip (NovaSeq X Series Sequencing v1.0)

The Load to Library Tube Strip (NovaSeq X Series Sequencing v1.0) step allows you to scan the library tube strip barcode into Clarity LIMS. Then, you can place the working pools into the library tube strip used in the NovaSeq X Series run. This step also does the following actions:

- Validates the run setup and analysis information.
- Generates the sample sheet file.
- Creates a planned run on ICA, depending on the selected run mode.

The following automations are configured on this step:

- Validate Input Count and Validate Analysis Configurations and Register Step Started
- Validate Library Tube Strip Barcode
- Validate Run Setup and Create Planned Run
- Set Next Step
- Register Step Completed

Validate Input Count and Validate Analysis Configurations and Register Step Started

Automatically triggered on entry to the step, this automation completes the following actions:

- Checks that the number of input pools is the same as the number of tubes in the library tube strip.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
script:validateSampleCount -min 8 -max 8
```

- Performs basic checks on the secondary analysis configuration of the samples in the same planned run. The following checks are included in this script:
  - Secondary analysis configuration of samples in a planned run is within maximum configuration limit.
  - Samples in a planned run have the same type of analysis (eg, Cloud or Local).
  - Samples in the same pools that have the same secondary analysis (eg, Illumina DRAGEN Enrichment) also have the same analysis settings (eg, Map/Align Output Format = CRAM) and the same analysis version (eg, v3.8.4).

```
/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/novaseqxser
ies-automation.jar script:validate_physical_logical_configurations -i
{stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -m
PerRun script:validate_analysis_config -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} -m PerRun &&
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar script:validate_same_analyte_udf_value_for_
all_inputs -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} -f 'Run Mode' -perPool false
```

- Registers the start time of the step by publishing messages to CLPA through ICA.

For Research Use Only. Not for use in diagnostic procedures.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s 'com/illumina/upa/scripts/common/step_started.groovy'
```

Validate Library Tube Strip Barcode Automation

Automatically triggered on the Placement screen, this automation validates the library tube strip barcode to make sure that it conforms to the barcode mask LC[0-9]{7}-LC1.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:validate_output_containers -l {compoundOutputFileLuid1} -r 'Library 8-tube Strip:LC[0-9]{7}-LC1' -max 1
```

Validate Run Setup and Create Planned Run Automation

Automatically triggered after a selection on the Record Details screen, this automation completes the following actions:

- Validates the parameters entered on the Record Details screen. These parameters are used to set up the run, generate the sample sheet file, and create the planned run in ICA, depending on the run mode.
  - Run Name can only contain alphanumeric, dash, underscore, or period characters. Do not use spaces.

    ```
    if (!step.::Run Name::.matches(::[a-zA-Z0-9-_]+[a-zA-Z0-9-_. ]*::)) { fail(::Run Name contains prohibited characters. Please check to make sure Run Name only contains alphanumeric characters, spaces, dashes and underscores. Run Name must start with alphanumeric, a dash or an underscore::); }
    ```

  - Run Name cannot exceed 255 characters.

    ```
    if (step.::Run Name::.length() > 255) { fail(::Run Name shall not exceed 255 characters.::); };
    ```

- Checks the Index 1 Cycles and Index 2 Cycles field values. If Index 2 Cycles is greater than 0, the Index 1 Cycles value must be greater than 0 or an error can occur.

```
if (step.::Index 2 Cycles:: > 0 && step.::Index 1 Cycles:: == 0) { fail(::Index 1 Cycles cannot be zero if Index 2 Cycles is non-zero::); };
```

- Generates the sample sheet. If you are in Cloud run mode, the automation creates the planned run in ICA. The sample sheet is attached to the step.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/novaseqxseries-automation.jar script:generate_sample_sheet -i {stepURI:v2} -u {username} -p {password} -c {compoundOutputFileLuid0} -l {compoundOutputFileLuid1}
```

There is a backend validation of the sample sheet content in ICA. This validation makes sure that the sample sheet is valid to set up a Local mode run and that it is ready for use with DRAGEN applications.

For more information on sample name character restrictions, refer to *NovaSeq X Series Integration v1.1 Validation and Troubleshooting* on page 86.

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation completes the following actions:

- Copies the Run Mode from input to output.
- Sets the next step for samples to ADVANCE, advancing them to the AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0) step.

```
output.::Run Mode:: = input.::Run Mode::; nextStep = ::ADVANCE::
```

Register Step Completed Automation

Automatically triggered on exit of the step, this automation registers the completion time of the step by publishing messages to CLPA through ICA.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s
'com/illumina/upa/scripts/common/step_completed.groovy'
```

Master Step Fields

The following table shows the master step fields that are configured on the Load to Library Tube Strip (NovaSeq X Series Sequencing v1.0) step. These fields are required for sample sheet generation and planned run creation in ICA.

Table 23 Clarity LIMS Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Cloud Run ID	Text	Read Only	Hidden Used for creating a planned run in ICA. Do not remove, rename, or modify this field as it can cause the integration to break.
Index 1 Cycles	Numeric Dropdown	Required Field Custom Entries	Range = 0–20 Presets • 0 • 6 • 8
Index 2 Cycles	Numeric Dropdown	Required Field Custom Entries	Range = 0–20 Presets • 0 • 6 • 8
Output Folder	Text		
Read 1 Cycles	Numeric Dropdown	Required Field Custom Entries	Range = 1–251 Presets • 51 • 101 • 151
Read 2 Cycles	Numeric Dropdown	Required Field Custom Entries	Range = 0–251 Presets • 51 • 101 • 151
Run Mode	Text Dropdown	Required Field	Presets • Local • Cloud
Run Name	Text	Required Field	

User Interaction

For more information, refer to the Load to Library Tube Strip (NovaSeq X Series Sequencing v1.0) step section in *NovaSeq X Series Integration v1.1 Validation and Troubleshooting* on page 86.

Step 5: AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0)

The AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0) step is fully automated. The integration starts the step, adds samples to the Ice Bucket, and completes the step. Data from the run is parsed back to Clarity LIMS. In this step, pooled samples in the library tube strip are sequenced on the NovaSeq X Series instrument.

The following automations are configured on this step:

- Register Step Started & Register NovaSeq X Run & Register NovaSeq X Run Association & Register NovaSeq X RunStarted
- Register NovaSeq X RunCompleted & Register Step Completed

Register Step Started & Register NovaSeq X Run & Register NovaSeq X Run Association & Register NovaSeq X RunStarted Automation

Automatically triggered at the beginning of the step, this automation completes the following actions:

- Registers the start time of the step.
- Registers the NovaSeq X Series sequencing run configuration.
- Registers the association of the sequencing run with the samples by linking the Instrument Run ID with the sample ID.
- Registers the time that the sequencing run started.

This automation completes these actions by publishing messages to CLPA through ICA.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s
'com/illumina/upa/scripts/common/step_started.groovy' &&
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s
'com/illumina/upa/scripts/novaseqxseries/register_novaseqxseries_run.groovy' &&
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s
'com/illumina/upa/scripts/novaseqxseries/novaseqxseries_associate_seqrun.groovy'
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
```

```
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s
'com/illumina/upa/scripts/novaseqxseries/novaseqxseries_seqrun_started.groovy'
```

Register NovaSeq X RunCompleted & Register Step Completed Automation

Automatically triggered on exit of the step, this automation completes the following actions:

- Registers the sequencing run completion time, run status, and run metrics. This script requires the Cloud Run ID custom field from the Load to Library Tube Strip (NovaSeq X Series Sequencing v1.0) step.
- Registers the completion time of the step.

This automation completes these actions by publishing messages to CLPA through ICA.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s
'com/illumina/upa/scripts/novaseqxseries/novaseqxseries_seqrun_completed.groovy'
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s
'com/illumina/upa/scripts/common/step_completed.groovy'
```

Master Step Fields

The following tables show the master step fields that are configured on the AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0) step.

Table 24 Clarity LIMS Master Step Field Information

Field Name	Source File	Corresponding Field in Source File	Additional Information
Current Cycle	`RunCompletionStatus.xml`	CompletedReads	Visible on Record Details screen. Calculated based on the CompletedReads field.

Field Name	Source File	Corresponding Field in Source File	Additional Information
Current Read	`RunCompletionStatus.xml` `RunParameters.xml`	CompletedReads PlannedReads	Visible on Record Details screen. Calculated based on the CompletedReads field in `RunCompletionStatus.xml` and PlannedReads field in `RunParameters.xml`.
Flow Cell Expiration Date	`RunParameters.xml`	ExpirationDate	Visible on Record Details screen. Corresponds to the value in the ExpirationDate field under the ConsumableInfo field for FlowCell Type.
Flow Cell ID	`RunParameters.xml`	SerialNumber	Visible on Record Details screen. Corresponds to the value in the SerialNumber field under the ConsumableInfo field for FlowCell Type.
Flow Cell Lot Number	`RunParameters.xml`	LotNumber	Visible on Record Details screen. Corresponds to the value in the LotNumber field under the ConsumableInfo field for FlowCell Type.
Flow Cell Part Number	`RunParameters.xml`	PartNumber	Visible on Record Details screen. Corresponds to the value of the PartNumber field under the ConsumableInfo field for FlowCell Type.
Flow Cell Side	`RunParameters.xml`	Side	Visible on Record Details screen.
Flow Cell Type	`RunParameters.xml`	FlowCellType	Visible on Record Details screen.

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Field Name	Source File	Corresponding Field in Source File	Additional Information
Instrument Control Software Version	`RunParameters.xml`	SystemSuiteVersion	Visible on Record Details screen.
Instrument ID	`RunParameters.xml`	InstrumentSerialNumber	Visible on Record Details screen.
Instrument Type	`RunParameters.xml`	InstrumentType	Visible on Record Details screen.
Output Folder	`RunParameters.xml`	OutputFolder	Visible on Record Details screen.
Library Tube Barcode	`RunParameters.xml`	SerialNumber	Visible on Record Details screen. Corresponds to the value of the SerialNumber field under the ConsumableInfo field for SampleTube Type.
Library Tube Lot Number	`RunParameters.xml`	LotNumber	Hidden Do not remove, rename, or modify this field as it can cause the integration to break. Corresponds to the value of the LotNumber field under the ConsumableInfo field for SampleTube Type.
Run End Time	`RunCompletionStatus.xml`	RunEndTime	Hidden Do not remove, rename, or modify this field as it can cause the integration to break.
Run Name	`RunParameters.xml`	ExperimentName	Visible on Record Details screen.

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Table 25 Other Clarity LIMS Master Step Fields

Field Name	Value	Additional Information
Run Status	Presets • RunStarted • RunCompletedSuccessfully • RunAbortedByUser • RunErroredOut Set by the SIS integration service.	Visible on Record Details screen.
Sequencing Log	Set by the SIS integration service during the sequencing run.	Visible on Record Details screen.
BaseSpace Run ID	Received from the SQS message in the SIS integration service.	Hidden Do not remove, rename, or modify this field as it can cause the integration to break.
ICA Project ID	Received from the SQS message in the SIS integration service.	Hidden Do not remove, rename, or modify this field as it can cause the integration to break.
Instrument Run ID	Received from the SQS message in the SIS integration service.	Hidden Do not remove, rename, or modify this field as it can cause the integration to break.
Run Start Time	Received from the SQS message in the SIS integration service.	Hidden Do not remove, rename, or modify this field as it can cause the integration to break.

Global Fields

The following global custom fields are used to capture the run metrics in Clarity LIMS:

- % Bases >=Q30 R1
- % Bases >=Q30 R2

- % Error Rate R1
- % Error Rate R2
- Yield (Gb) R1
- Yield (Gb) R2
- Reads PF R1
- Reads PF R2
- %PF R1
- %PF R2
- % Aligned R1
- % Aligned R2
- % Phasing R1
- % Phasing R2
- % PrePhasing R1
- % PrePhasing R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- Cluster Density R1
- Cluster Density R2

At the end of the step, the pools of samples are automatically removed from the step. The step completes automatically when Run Status is RunCompletedSuccessfully.

How the Integration Works

The following information summarizes how the NovaSeq X Series integration works.

- After the Validate Run Setup and Create Planned Run automation is triggered on the Load to Library Tube Strip (NovaSeq X Series Sequencing v1.0) step, the run parameters entered in the Run Details screen are sent to ICA. The analysis configuration parameters from the selected ACTs are also sent to ICA. ICA validates the run and analysis configuration. If the validation fails, ICA sends an error message to Clarity LIMS. If the validation passes, ICA generates the sample sheet and sends it back to Clarity LIMS. ICA also creates the planned run based on the selected run mode (eg, Local or Cloud).
  - For the Local run mode, the sample sheet is generated and stored in Clarity LIMS. This sample sheet contains the run and analysis configuration information required to start the run on the NovaSeq X Series instrument.

  - For the Cloud run mode, the planned run is created in ICA. The planned run contains the run and analysis configuration information required to start the run on the instrument. The sample sheet is generated and stored in Clarity LIMS for reference purposes.
- When the sequencing run starts on the instrument, the NovaSeq X Series Control Software notifies BaseSpace Sequence Hub. BaseSpace Sequence Hub monitors the run and uses the Stratus Integration Service (SIS) to notify Clarity LIMS. The events are processed and the integration service retrieves the run information from BaseSpace Sequence Hub. This information is used to populate the custom fields in the AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0) step.
- Other run events follow the same information flow. When sequencing is complete, the control software uploads the sequencing run data (primary metrics) and associated files to ICA. Then, Clarity LIMS retrieves the primary metrics and uses them to populate the fields in the Sample Details table (eg, % Error Rate R1). The custom fields (eg, Run Status, Current Read, and so on) on the AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0) step are updated using the run related information. If the sequencing run is successfully completed, the step automatically completes.
- The analysis results are not tracked. You must log in to BaseSpace Sequence Hub to retrieve the cloud analysis results. For local analysis, the secondary analysis results are found in the external storage configured in Illumina Run Manager. You can find this storage information under External Storage and Analysis Results.
- If a planned run with the same sample name and project name (case-insensitive) has been created previously in ICA, the sample sheet generated from the Validate Run Setup and Create Planned Run automation can reflect the original case of the previous sample name. This can cause validation errors for analysis configurations with sample-level settings. To resolve this issue, change the sample name or the project name on Clarity LIMS and run the automation again.

Start a Sequencing Run on NovaSeq X Series Instrument

The following sections outline the steps to start a Clarity LIMS cloud or local run on the NovaSeq X Series instrument.

Run Mode Configuration

1. In the NovaSeq X Series Control Software, log in to the instrument using an account that has permissions for configuring settings.
2. From the menu, select Settings.
3. From Settings, select Cloud settings.
4. Under Run Settings, select the Cloud run storage checkbox.
5. For Hosting location, select the applicable location from the drop-down options.

6. [Optional] For Private domain name, enter the domain name according to the workgroup that was configured with the NovaSeq X Series integration package.
7. Select Save.

Start a Sequencing Run in Local Run or Cloud Run Modes

You can start the sequencing run in BaseSpace Sequence Hub for both the Local and Cloud modes. Make sure that you log in using the account that was used during the NovaSeq X Series integration package setup. You must also import the sample sheet created from the Load to Library Tube Strip (NovaSeq X Series Sequencing v1.0) step.

Start a Sequencing Run in Local Run Mode

1. Log in with the BaseSpace Sequence Hub account and select the workgroup used during the NovaSeq X Series integration package installation.
2. Import the sample sheet created in the Load to Library Tube Strip (NovaSeq X Series Sequencing v1.0) step. Refer to information on creating a local run in the *NovaSeq X Plus Product Documentation* on the NovaSeq X Plus Sequencing System support site.
3. When the sample sheet is selected, the contents are validated. After the validation passes, select Next.
4. Select Save As Planned.
   Run and analysis data is parsed and auto-populates in the list of runs. For more information on planning a NovaSeq X Series run, refer to the Plan a NovaSeq X Series Run section of the BaseSpace Sequence Hub Online Help at help.basespace.illumina.com and the *NovaSeq X Plus Product Documentation* on the NovaSeq X Plus Sequencing System support site.
5. Start the run. Refer to information on starting a planned run in the *NovaSeq X Plus Product Documentation* on the NovaSeq X Plus Sequencing System support site.

Start a Sequencing Run in Cloud Run Mode

To start a sequencing run in cloud run mode, refer to information on starting a planned run in the *NovaSeq X Plus Product Documentation* on the NovaSeq X Plus Sequencing System support site.

Components Installed

The following sections describe the components (files, properties, reagent categories / label groups, reagent kits, and containers) that are installed by default as part of this integration.

Reagent Kits

- Buffer Cartridge
- Lyophilization Cartridge
- NaOH
- Reagent Cartridge
- Resuspension Buffer (RSB)

- TT2

Container Types

- Library 8-Tube Strip
- Tube

This integration supports the library 8-tube strip with the barcode in the LC[0-9]{7}-LC1 format (eg, LC1234567-LC1).

Control Types

- PhiX v3

Rules and Constraints

- The workflow configuration contains several validation checks. To make sure that the calculations work properly, it is important that you do not disable any of this validation logic. The validation checks determine the following information:
  - Which samples, and how many, can enter each step together.
  - Which samples, and how many, can be pooled together.
- All submitted samples must have an associated secondary analysis that is configured using the analysis configuration template (ACT). The ACT must be configured on BaseSpace Sequence Hub before starting the Assign Analysis Configuration Template step. The ACT names must be unique.
- The library tube strip barcode must be unique. There must not be multiple library tube strip containers with the same name in the system.
- Reagent labels, or indexes, must be unique.
- One library pool can only contain one library or control with no label/index.
- The AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0) step must not be manually started or completed. This step is fully automated and the sequencing service does not update samples correctly if they have been manually started.
- For the automated run to start successfully, you must select Validate Run Setup and Create Planned Run in the Load to Library Tube step.

NovaSeq X Series Integration v1.1 Validation and Troubleshooting

This section explains how to validate the installation of the Illumina NovaSeq X Series Integration Package v1.1.

The validation process involves the following actions:

- Running samples through the Library Prep Validation v2.3.1 workflow.

  - The workflow contains a single-step protocol that models the library prep required to produce libraries tagged with index sequences. At the end of the step, these libraries are routed to NovaSeq X Series Sequencing v1.0 workflow.
- Running libraries through the NovaSeq X Series Sequencing v1.0 workflow validates the following items:
  - Successful sequential step advancement of samples through the steps of the workflow.
  - Automatic validation of run setup information before sample sheet generation or planned run creation.
  - Automated sample sheet generation. This sample sheet is used to start the sequencing run on the NovaSeq X Series Control Software through the Local run mode.
  - Automated creation of a planned run in Illumina Connected Analytics (ICA). The control software retrieves the planned run. The run is started through the Cloud run mode.
  - Automated tracking of the NovaSeq X Series sequencing run and parsing of run statistics into Clarity LIMS.

The validation steps assume that the following conditions have been met:

- NovaSeq X Series Integration Package v1.1 is installed and you have imported the default Clarity LIMS configuration.
- Analysis configuration templates (ACTs) are created in BaseSpace Sequence Hub (BaseSpace Sequence Hub) for your run. Make sure that the index adapter kit label group is created in Clarity LIMS before selecting it in the ACT. This same label group is used in the Run Library Prep Validation v2.3.1 step. For more information on creating a reagent label group, refer to *Add and Configure Labels and Label Groups* in the Clarity LIMS Core Documentation. For the adapter sequences for your library prep kits, refer to *Illumina Adapter Sequences*.

Analysis Configuration Template Creation in BaseSpace Sequence Hub

You must create the Analysis Configuration Templates (ACTs) that are required for configuring secondary analysis in the NovaSeq X Series Sequencing v1.0 workflows. Create and delete ACTs in BaseSpace Sequence Hub. For instructions, refer to the BaseSpace Sequence Hub online help.

Activate Workflow, Create Project, Add and Assign Samples

The following steps set up Clarity LIMS in preparation for running samples through the Library Prep Validation v2.3.1 and NovaSeq X Series Sequencing v1.0 workflows.

1. On the Configuration tab, under Workflows, activate both the Library Prep Validation v2.3.1 and NovaSeq X Series Sequencing v1.0 workflows.
2. On the Projects and Samples screen, create a project and add samples to it.

> Only use alphanumeric, dash, or underscore characters in the submitted sample names. Invalid characters can cause a sample sheet validation failure in the Load to Library Tube Strip step.

3. Assign the samples to the Library Prep Validation workflow.

Library Prep Protocol: Library Prep Validation v2.3.1

This single-step protocol models the library prep required to produce libraries tagged with index sequences that are ready for the NovaSeq X Series Sequencing v1.0 workflow.

Run Library Prep Validation v2.3.1

1. In Lab View, locate the Library Prep Validation v2.3.1 protocol.
   Samples are queued for the Library Prep Validation v2.3.1 step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, under Label Options, select the label groups that you want to use.
   The label group, or index adapter kit, selected must be the same as the Index Adapter Kit selected in the ACT that is being used.
4. Select Begin Work.
5. On the Placement screen, complete the following actions:
   a. In the Placed Samples area on the right, in the Container field, scan or enter the barcode of the destination container.
   b. Select the samples from the Samples to be Placed area on the left, and drag them to the container wells on the right.
   c. Select Add Labels.
6. On the Add Labels screen, the Labels to Add list contains labels from the label group selected in step 3.
   a. Select labels on the left, and drag them to the container on the right to assign them to placed samples.
   b. Select Record Details.
7. On the Record Details screen, complete the following actions:
   a. In the Sample Details table, enter values for Molarity (nM). Type values individually or copy and paste to enter multiple values from a spreadsheet.

   > The Molarity (nM) values are used in the Calculate Volume automation script in the Make Bulk Pool (NovaSeq X Series Sequencing v1.0) step. If these values are not entered, the automation script does not work properly.

   b. The Sequencing Instrument column indicates the instrument to be used for the sequencing run. Select NovaSeq X Series.
   c. Populate the fields in the Sequencing Instrument column using one of the following methods:
      - To populate fields individually for each sample, select from the drop-down list in each row.

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- To populate all fields at one time, copy and paste from a spreadsheet.
- Alternatively, select inside the table and press Ctrl + A to select all rows. At the top of the table, select Sequencing Instrument and then select NovaSeq X Series from the adjacent drop-down list. Select Apply.

If working with many samples, copy and paste to insert multiple values from a spreadsheet as follows.

a. Select Copy Samples to copy the sample information from Clarity LIMS to the Clipboard.
b. Open a blank Excel document and paste the contents of the clipboard into the spreadsheet.
c. In the spreadsheet, enter the Molarity (nM) and Sequencing Instrument values. Select the entire spreadsheet including the headers (Ctrl + A), and copy (Ctrl + C) the information to the Clipboard.
d. Within Clarity LIMS, select the Paste Samples button. Paste the contents of the clipboard (Ctrl + V) into the Update Table window and then select Update to paste the contents into the Sample Details table.

8. Select Next Steps.
   This selection triggers the Set Next Steps automation, which sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step.
9. On the Assign Next Steps screen in the Samples table, the Next Step value for all samples is prepopulated with Remove from workflow.

   Do not change this value. If Next Step is not set to Remove from workflow, the routing script does not route samples correctly.

10. Select Finish Step.
    On exit from the step, the Routing Script automation is triggered. This automation assigns samples to the first step of the NovaSeq X Series Sequencing v1.0 workflow, Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.0).

Protocol: NovaSeq X Series Sequencing v1.0

The NovaSeq X Series Sequencing v1.0 protocol consists of the following steps:

- Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.0)
- Make Bulk Pool (NovaSeq X Series Sequencing v1.0)
- Dilute and Denature (NovaSeq X Series Sequencing v1.0)
- Load To Library Tube Strip (NovaSeq X Series Sequencing v1.0)
- AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0)

Each step contains a script to register the start time (upon step entry) and the time that the step is completed (upon step exit). This information is published to CLPA through the ICA LINK service. These scripts are used only for CLPA support and can be incorporated as part of an automation that performs other functions.

Step 1: Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.0)

1. In Lab View, locate the NovaSeq X Series Sequencing v1.0 protocol. The samples are queued for the Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.0) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work to start the Validate Sample Names and Retrieve Analysis Configuration Template List and Register Step Started automation.
   The automation checks that the sample names do not use restricted characters and are within character limits and retrieves the list of ACTs that you can select. The automation also registers the start time of the step by publishing messages to CLPA through the ICA LINK service. The part of the script for start time registration is used only for CLPA support.
4. Select the applicable ACT to assign to the samples. The index adapter kit specified by the ACT must correspond with the label group used in *Library Prep Protocol: Library Prep Validation v2.3.1* on page 88.
5. [Optional] Under Step Details, select Retrieve ACT Information to trigger the Retrieve ACT Information automation.
   This automation retrieves ACT information (eg, Library Prep Kit, Index Adapter Kit, Reference Genome, and so on) and populates the fields in Clarity LIMS. These details are saved to the `ACTMetadata.csv` file that you can download. If you are not sure of the analysis configuration before starting the sequencing and analysis run, refer to the details in the file.
6. Select Next Steps to assign the ACT to the samples.
   This action triggers the Validate Reagent Labels and Apply Selected ACT to Samples and Set Next Step automation. This automation validates that the indexes applied to the libraries are valid for the selected ACT and assigns samples to it. All samples added to the Ice Bucket in 2 are assigned to this ACT.
7. Select Finish Step.

To use multiple ACTs in a single run, repeat 2–7 for each ACT. For step 2, only add samples that are being assigned to that ACT into the Ice Bucket.

Step 2: Make Bulk Pool (NovaSeq X Series Sequencing v1.0)

1. In Lab View, locate the NovaSeq X Series Sequencing v1.0 protocol. The samples are queued for the Make Bulk Pool (NovaSeq X Series Sequencing v1.0) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.

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You can also add control samples by selecting them from the Available control samples drop-down list and selecting Add.

3. On the Ice Bucket screen, select Begin Work.
4. Create a pool of samples as follows.
   a. On the Pooling screen, create a pool by dragging samples into the Pool Creator.
   If a control sample was added, it appears under the Sample List and can be pulled into the pool that needs the control.
   b. Enter a name for your pool or accept the default name (Pool #1).
   c. [Optional] If multiple pools are required, select the plus sign (+) next to Pool Creator to create a pool.
   d. [Optional] To remove a pool, select the X in the top right corner of the pool.
   e. Select Record Details to trigger the Validate Analysis Configurations automation.
   This automation performs the following checks on the analysis configuration for each pool:
      - Pooled samples are within the maximum configuration limit.
      - Pooled samples have the sample type of analysis (Cloud or Local).
      - Pooled samples that have the same secondary analysis also have the same analysis version and settings.
5. On the Record Details screen, navigate to the Reagent Lot Tracking section to track the lot information used in the step.
6. [Optional] Create a new lot. For more information, refer to *Add and Configure Reagent Kits and Lots* in the Clarity LIMS LIMS Core Functionality documentation.
7. In the Step Details area, complete the following required fields:
   - Number of Lanes to Sequence—Used in volume calculations, to make sure that the volumes are sufficient for the number of times the pool is sequenced. This field is applied to all pools in the step.
   - Final Loading Concentration (pM)—The final loading concentration of the pool in the flow cell.
   - Final Loading Volume (µl)—The final loading volume of the pool in the flow cell. This field is prepopulated with the configured default value (200 µl), but can be edited if more volume is required.
   - Minimum Per Sample Volume (µl)—The minimum volume for each sample. This field is prepopulated with the configured default value (2 µl) and can be edited. If the per sample volume is below the value set in this field, the Calculate Volumes script applies a rounding factor to affected samples so that the volume reaches the minimum volume.
   - % PhiX (2.0nM) Spike-In—The value entered in this field is used to calculate the volume of PhiX spike-in required.
   - Flowcell Type—This is a read-only field configured with the default value of B3.
8. Select Calculate Volumes to trigger the Calculate Volumes automation.

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This automation calculates the volumes required for each library to form a pool that has the concentration and volume specified in the Step Details field. This automation is applied to control samples when they are indexed and molarity is specified. It also generates the calculation file in a CSV format and attaches it to the step. You can select the file name to download it.

9. In the Sample Details table, select the pool next to the sample name to view details on the pool composition.
10. Select Next Steps to trigger the Set Next Step automation.
    This automation sets the next step for samples to ADVANCE, which moves them to the Dilute and Denature (NovaSeq X Series Sequencing v1.0) step.
11. Select Finish Step.

Step 3: Dilute and Denature (NovaSeq X Series Sequencing v1.0)

1. In Lab View, locate the NovaSeq X Series Sequencing v1.0 protocol. The pool of samples queued for the Dilute and Denature (NovaSeq X Series Sequencing v1.0) displays.
2. Add the pool to the Ice Bucket and select View Ice Bucket.
3. [Optional] On the Ice Bucket screen, set the number of derivatives to create (placed into the library tube strip) and select Begin Work.
   - On entry to the step, the Validate Inputs Flowcell Type and Register Step Started automation is triggered. This automation makes sure that the configured flow cell type is valid. The automation also registers the start time of the step by publishing messages to CLPA through the ICA LINK service. The portion of the script used for start time registration is used only for CLPA support.
   - On entry to the Record Details screen, the Calculate Volumes automation is triggered.
     This automation sets the following values:
     - BP Aliquot Volume (µl)
     - RSB Volume (µl)
     - NaOH Volume (µl)
     - TT2 Volume (µl)
     - The automation also generates the calculation file (CSV) and attaches it to the step. This file contains information about the volume of RSB, NaOH, and TT2 to add per working pool.
4. On the Record Details screen, the Reagent Lot Tracking section tracks the NaOH, Resuspension Buffer, and TT2 reagents used in the step. To add and activate reagent lots, do as follows.
   a. In a new browser tab, open an additional instance of Clarity LIMS.
   b. Navigate to the Consumables configuration screen.
   c. Select one of the reagent kits previously listed (eg, NaOH) and select New Lot.
   d. Enter the lot details.
   e. Select Save.

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f. Set the Status of Reagent Lot toggle switch to Active.

g. Repeat this process to add and activate the remaining reagent lots.

h. Return to your original working browser tab and refresh the page.

5. On the Record Details screen, perform the following actions:

a. In the Reagent Lot Tracking section, select from the active lots displayed in each drop-down list.

b. In the Sample Details table, make sure that the BP aliquot, RSB, NaOH, and TT2 reagent volume values are populated. The script sets these values and they cannot be edited.

c. [Optional] In the Sample Details table, select the pool icon to view details of the working pool composition.
The working pool number is appended to the bulk pool name so that you can identify which working pools are derived from the same bulk pool.

6. In the Files area, select the Calculation File (CSV) to open it and view details on the RSB, NaOH, and TT2 volumes to add per working pool.

7. Select Next Steps.

8. Select Finish Step.

Step 4: Load to Library Tube Strip (NovaSeq X Series Sequencing v1.0)

1. In Lab View, locate the NovaSeq X Series Sequencing v1.0 protocol. The pool of samples are queued for the Load to Library Tube Strip (NovaSeq X Series Sequencing v1.0) step.

2. Add the pools to the Ice Bucket and select View Ice Bucket.

3. On the Ice Bucket screen, select Begin Work to trigger the Validate Analysis Configurations and Register Step Started automation.
This automation performs the following basic checks on the analysis configuration for each pool:

- Pooled samples are within the maximum configuration limit.
- Pooled samples have the sample type of analysis (Cloud or Local).
- Pooled samples that have the same secondary analysis also have the same analysis version and settings.

The automation also registers the start time of the step by publishing messages to CLPA through the ICA LINK service. The portion of the script used for start time registration is used only for CLPA support.

4. On the Placement screen, do as follows.

a. Drag the pool into the Library 8-tube Strip in the Placed Samples area on the right.

b. Scan or type the barcode of the Library 8-tube Strip into the Library 8-tube Strip container name field.

c. Select Record Details.

After exiting the Placement screen, the Validate Library Strip Tube Barcode automation makes sure that the library tube strip barcode conforms to the barcode mask LC[0-9]{7}-LC1.

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5. The fields displayed on the Record Details screen are used to create the planned run and generate the sample sheet.
   The analysis related information for the planned run is from the ACT associated with the samples and no further analysis configuration is required. Refer to the following table for details.

   Table 26 Fields Displayed on Record Details Screen of Load to Library Tube Strip (NovaSeq X Series Sequencing v1.0) Step

Field	Value
Run Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. No spaces.
Run Mode	The selected Run Mode must correspond to the ACT type. Presets • Local[1] • Cloud[2]
Read 1 Cycles	Presets • 151 • 101 • 51 • Type a custom value
Read 2 Cycles	Presets • 151 • 101 • 51 • Type a custom value
Index 1 Cycles	Presets • 0 • 6 • 8 • Type a custom value[3]
Index 2 Cycles	Presets • 0 • 6 • 8 • Type a custom value[3]

   [1] The Local run mode generates the sample sheet. The Run/Analysis configuration is imported manually to the NovaSeq X Series instrument through the sample sheet. The sample sheet is downloaded and imported manually by the user on NovaSeq X Control Software to start the run. The downstream secondary analysis is done using the onboard DRAGEN module.
   [2] The Cloud run mode sends the Run/Analysis configuration to ICA. This configuration is downloaded to the NovaSeq X Series instrument through ICA to start the run. The downstream secondary analysis is done in the cloud.

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[3] The custom value must correspond to the longest index sequence of the samples in the pools in the library tube strip. Otherwise, the planned run creation fails and an error message displays.

6. Select Validate Run Setup and Create Planned Run to trigger the automation script. The script performs the following actions:
   - Validates the parameters entered on the Record Details screen.
   - Creates the planned run and sends it to ICA when the Run Mode is Cloud.
   - Generates the sample sheet and attaches it to the placeholder in the Files area on the Record Details screen for all Run Mode types.
7. Select Next Steps.
   On the Assign Next Steps screen, the next step for the pooled samples is set to the AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0) step.
8. Select Finish Step to advance the pooled samples to the AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0) step. For more information on how to start the sequencing run for different run modes, refer to *NovaSeq X Series Integration v1.1 Configuration and User Interaction on page 56*.
9. To use multiple ACTs in a single run, repeat steps 1–3 for each ACT. The pool of samples (for different ACTs) are then queued for the Load to Library Tube Strip (NovaSeq X Series Sequencing v1.0) step to be added to the Ice Bucket.

Step 5: AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0)

This protocol contains the AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0) step. This step is fully automated.

Do not add samples to the Ice Bucket or start or complete the AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0) step. The integration does this automatically.

The integration starts the step automatically and data from the run is parsed back into Clarity LIMS. User interaction is not required, but you can review the stages of the step in Clarity LIMS.

Review Run Data

Read summary metrics are recorded for the library pool. After the run is complete, open the step and review these metrics in the Step Details section and the Sample Details table.

Step Details Section

The following values populate the master step fields:

- Run Name
- Run Status
- Output Folder

- Current Read
- Current Cycle
- Library Tube Barcode
- Flow Cell ID
- Flow Cell Side
- Flow Cell Type
- Flow Cell Part Number
- Flow Cell Lot Number
- Flow Cell Expiration Date
- Instrument ID
- Instrument Type
- Instrument Control Software Version
- Sequencing Log

Sample Details Table

Summary metrics populate the following global custom fields:

- % Bases >=Q30 R1
- % Bases >=Q30 R2
- % Error Rate R1
- % Error Rate R2
- Yield (Gb) R1
- Yield (Gb) R2
- Reads PF R1
- Reads PF R2
- %PF R1
- %PF R2
- % Aligned R1
- % Aligned R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2

- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- Cluster Density R1
- Cluster Density R2

Troubleshooting

If an automation trigger does not appear to run its corresponding scripts, refer to the *Troubleshooting Automation* section in the Clarity LIMS API & Database documentation.

Automated Step Does Not Start

If the AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0) step does not start, Clarity LIMS is likely not receiving sequencing run events from BaseSpace Sequence Hub correctly. Check for the following issues:

1. Make sure that the NovaSeq X Control Software is configured properly as follows.
    a. Before you start the run, in the NovaSeq X Control Software, select the appropriate BaseSpace Sequence Hub region in the Hosting location drop-down list.
    b. Under Run Settings, check the boxes next to Cloud run monitoring and Cloud run storage.

If there are issues after using the configuration settings on the NovaSeq X Control Software, contact Illumina Support.

Automated Step Starts, But Does Not Complete

If the AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0) step starts, but does not complete, do as follows.

1. Log in to the default user account and use one of the following methods to open the in progress step in Clarity LIMS:
    a. In Lab View, find the step in the Recent Activities pane.
    b. Search for the step in Clarity LIMS using the library tube strip barcode as the search term.
2. On the Record Details screen, the Sequencing Log multiline text field contains logging information. If unable to reach the Record Details screen, or if the Sequencing Log field does not contain enough information to resolve the issue, contact Illumina Support. Provide the relevant information from the troubleshooting steps already performed.

Validate Analysis Configuration Automation Check Fails

The Validate Analysis Configuration automation check is in the Make Bulk Pool (upon pooling) and Load to Library Tube Strip (upon step entry) steps. If a failure happens, an error message displays and the step can be aborted, or you might be prevented from continuing the step. This check can fail for the following reasons:

- The analysis configuration after library pooling or during the planned run creation exceeds the configuration limit. Resolve this error as follows.
  - If the error occurs on the Make Bulk Pool step, reduce the number of analysis configurations for a pool. To reduce the number, reorganize the samples for the pooling step (eg, by pooling samples that have similar configurations together).
  - If the error occurs on the Load to Library Tube step, reduce the number of analysis configurations for the planned run by reorganizing the samples for multiple runs instead of a single run.
- ACTs of samples in the same pool or planned run must have the same run mode (Local or Cloud). Resolve this error as follows.
  1. Abort the step and remove samples with ACTs that have conflicting run modes from the Ice Bucket.
  2. Make sure that all remaining samples in the Ice Bucket have ACTs with the same run modes.
  3. Select Begin Work to continue the step.
- ACTs of samples in the same pool or planned run that have the same secondary analysis must have the same analysis version. Resolve this error as follows.
  - If the error occurs on the Make Bulk Pool step, pool the samples again. Make sure that samples with the same secondary analysis, but conflicting analysis versions, are in different pools.
  - If the error occurs on the Load to Library Tube step, reorganize the samples for the planned run. Make sure that samples with the same secondary analysis, but conflicting analysis versions, are in different runs.
- ACTs of samples in the same pool or planned run that have the same secondary analysis must have the same analysis settings. The analysis setting fields can differ for a different secondary analysis and are configured in BaseSpace Sequence Hub when the ACT is created. Resolve this error as follows.
  1. If the error occurs on the Make Bulk Pool step, pool the samples again. Make sure that samples with the same secondary analysis, but conflicting analysis settings, are in different pools.
  2. If the error occurs on the Load to Library Tube step, reorganize the samples for the planned run. Make sure that samples with the same secondary analysis, but conflicting analysis settings, are in different runs.

NovaSeq X Series Integration v1.1 Release Notes

These Release Notes describe the key changes to software components for the Clarity LIMS NovaSeq X Series Integration Package v1.1.

Compatibility

This version of the integration package is compatible with the following software:

- Clarity LIMS v6.2 and later
- NGS Extensions Package v5.23 and later
- Illumina Preset Protocols (IPP) v2.6.0 and later
- NovaSeq X Series Control Software v1.0.0

New Features

- Updated Java and third-party dependency libraries.
- Updated Groovy to v3.0.7.

Defects Fixed

- Restricted the scope for the JWT token used in the integration.
- Fixed issue where the SIS core service was not properly registered in systemctl.

Known Limitations

- This integration does not track the analysis step. You must log in to BaseSpace Sequence Hub to view the cloud analysis status and results. For local analysis, the secondary analysis results are located in the external storage configured in Illumina Run Manager.
- The Local run mode does not support custom library prep and index adapter kits.
- The Analysis Configuration Template (ACT) names must be unique.

Known Issues

- On the Make Bulk Pool step, the log displays a warning when the Calculate Volume automation is triggered and at least one pool consists of multiple inputs. This issue is caused by the output custom field being reset multiple times at the end of the automation. This issue does not affect the volume calculation.

NovaSeq X Series Integration v1.0 Configuration and User Interaction

The Illumina NovaSeq X Series Integration Package v1.0 supports the integration of Clarity LIMS to Illumina NovaSeq X series instruments. This integration supports Clarity LIMS v5.1 and later.

For instructions on validating and troubleshooting the Illumina NovaSeq X Series Integration, refer to *NovaSeq X Series Integration v1.0 Validation and Troubleshooting* on page 129.

The configuration provided in this integration has been established to support NovaSeq X Series lab processes. Any configuration changes to protocols or workflows (including renaming protocols, steps, and fields) could break the process.

Overview

The integration includes the following features:

- Preconfigured NovaSeq X Series Sequencing v1.0 workflow that maps to lab protocols and instrument runs.
- The following steps in the NovaSeq X Series Sequencing v1.0 preconfigured protocol:
  - Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.0)
  - Make Bulk Pool (NovaSeq X Series Sequencing v1.0)
  - Dilute and Denature (NovaSeq X Series Sequencing v1.0)
  - Load to Library Tube Strip (NovaSeq X Series Sequencing v1.0)
  - AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0)
- Automatic validation of run setup information before sample sheet generation or planned run creation.
- Automated generation of the sample sheet. This sample sheet is used to start the sequencing run on the NovaSeq X Series Control Software through Local run mode.
- Automated creation of a planned run on Illumina Connected Analytics (ICA). The NovaSeq X Series Control Software retrieves the planned run for use with the Cloud run mode.
- Automated tracking of the NovaSeq X Series sequencing run and parsing of run statistics into Clarity LIMS using the Stratus Integration Service (SIS).
- [Optional] Preconfigured Library Prep Validation v2.3.0 workflow used for validation purposes only. The workflow contains a single-step protocol that models the library prep required to produce libraries that are ready for the NovaSeq X Series Sequencing v1.0 workflow. For more information, refer to *NovaSeq X Series Integration v1.0 Validation and Troubleshooting* on page 129. The Library Prep Validation v2.3.0 workflow is available in the Illumina Preset Protocols (IPP) v2.5 package.
- [Optional] Clarity LIMS Product Analytics integration that can be used with ICA. The Clarity LIMS Product Analytics integration installation and configuration is required. For more information, refer to *Clarity LIMS Product Analytics Service Integration v1.1 Installation* and *Clarity LIMS Product Analytics Integration v1.1 Configuration and User Interaction* on page 2415.

Prerequisites and Assumptions

Analysis Configuration Template

The NovaSeq X Series Sequencing v1.0 workflow uses analysis configuration templates (ACTs) to configure secondary analysis for planned runs. Required ACTs must be created using BaseSpace Sequence Hub. The index adapter kit (the label group in Clarity LIMS) selected in the ACT is created in Clarity LIMS. The same label group must be used in the library preparation step. For more information, refer to *NovaSeq X Series Integration v1.0 Validation and Troubleshooting* on page 129.

Make sure that ACT names created in BaseSpace Sequence Hub do not have leading or trailing spaces. Otherwise, Clarity LIMS can have issues with recognizing the ACT names.

Samples

Samples must go through the library preparation and quantification process before they enter the NovaSeq X Series Sequencing v1.0 workflow. It is assumed that the following steps have completed before samples are assigned to the workflow:

- Samples have been accessioned into Clarity LIMS.
- Samples have been run through QC and library prep.
- Samples have the Molarity (nM) global field set to a value. This value is required for the Calculate Volumes automation in the Make Bulk Pool step.

For more information on sample accessioning, refer to *Sample Accessioning* and *Upload and Modify Samples* in the Getting Started section of the Clarity LIMS Core Functionality documentation.

You can assign samples to workflows automatically, using a routing script, or manually—from the Projects & Samples dashboard. Refer to *Assign and Process Samples* in the Clarity LIMS Core Functionality documentation.

Workflows, Protocols, and Steps

The NovaSeq X Series Integration Package v1.0 includes the following workflows:

- NovaSeq X Series Sequencing v1.0
- Library Prep Validation v2.3.0 (optional, but recommended for validation purposes)

The following table describes the protocols and steps included in these workflows.

Table 27 NovaSeq X Series Integration Package v1.0 Protocols and Steps

Protocol Name	Protocol Purpose	Protocol Steps
Library Prep Validation v2.3.0 Workflow		
Library Prep Validation v2.3.0	Included for validation purposes only, this protocol models the library prep steps required to advance samples to the NovaSeq X Series Sequencing v1.0 workflow. The protocol contains a single step - Library Prep Validation v2.3.0. After this step, a routing script sends the samples to the first step of the NovaSeq X Series Sequencing v1.0 workflow (Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.0)).	1. Library Prep Validation v2.3.0
NovaSeq X Series Sequencing v1.0 Workflow		

Protocol Name	Protocol Purpose	Protocol Steps
NovaSeq X Series Sequencing v1.0	Allows for the assignment of per sample values for the following properties: 1. Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.0) • The secondary analysis of samples is configured using the ACT. • The labels applied to the samples are validated against the selected ACT so that samples use valid labels. 2. Make Bulk Pool (NovaSeq X Series Sequencing v1.0) • The Calculate Volumes script assists with pooling samples and adding resuspension buffers and reagents. • The Validate Analysis Configurations automation validates the ACT of pooled samples to make sure that the secondary analysis configurations remain valid after pooling. 3. Dilute and Denature (NovaSeq X Series Sequencing v1.0) • The Calculate Volumes script helps denature and dilute samples from step 2 to the final loading concentration. 4. Load To Library Tube Strip (NovaSeq X Series Sequencing v1.0) • The library pools from step 3 are ready to be loaded to the NovaSeq X Series library tube strip. • Validates run and analysis information. • Generates the sample sheet and creates the planned run. 5. AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0) • The step is fully automated and starts and completes automatically after the sequencing run is started and completed on the instrument side. • The sequencing run metadata (eg, run configuration, primary run metrics, and so on) are recorded automatically. • The integration does this step	1. Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.0) 2. Make Bulk Pool (NovaSeq X Series Sequencing v1.0) 3. Dilute and Denature (NovaSeq X Series Sequencing v1.0) 4. Load To Library Tube Strip (NovaSeq X Series Sequencing v1.0) 5. AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0)

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Validation Workflow

The Library Prep Validation v2.3.0 workflow allows for validation of the system after installation is complete. For details, refer to *NovaSeq X Series Integration v1.0 Validation and Troubleshooting* on page 129.

Step Automations

The following table lists the step automations included in the NovaSeq X Series Sequencing v1.0 workflow. Automation details are provided in the documentation for each step.

For details on the validation workflow, refer to *NovaSeq X Series Integration v1.0 Validation and Troubleshooting* on page 129.

Table 28 NovaSeq X Series Sequencing v1.0 Workflow Step Automations

Protocol	Step	Automation
NovaSeq X Series Sequencing v1.0	1. Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.0)	Register Step Completed[1] Retrieve ACT Information Validate Reagent Labels and Apply Selected ACT to Samples and Set Next Step Validate Sample Names and Retrieve Analysis Configuration Template List and Register Step Started[2]
	2. Make Bulk Pool (NovaSeq X Series Sequencing v1.0)	Calculate Volumes Register Pools and Register Step Completed[1] Register Step Started[1] Set Next Step Validate Analysis Configurations
	3. Dilute and Denature (NovaSeq X Series Sequencing v1.0)	Calculate Volumes Register Step Completed[1] Set Next Step Validate Inputs Flowcell Type and Register Step Started[2]
	4. Load to Library Tube Strip (NovaSeq X Series Sequencing v1.0)	Register Step Completed[1] Set Next Step Validate Analysis Configurations and Register Step Started[2] Validate Library Tube Strip Barcode Validate Run Setup and Create Planned Run
	5. AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0)	Register NovaSeq X RunCompleted and Register Step Completed[1] Register Step Started and Register NovaSeq X Run and Register NovaSeq X Run Association and Register NovaSeq X RunStarted[1]

[1] These automations are required for Clarity LIMS Product Analytics support only.
[2] These automations are required for the NovaSeq X Series Sequencing v1.0 workflows and contain additional logic needed for Clarity LIMS Product Analytics support. If you would like to remove support for Clarity LIMS Product Analytics, contact Illumina Support.

All remaining automations are required for the NovaSeq X Series Sequencing v1.0 workflows.

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The Illumina Universal Sample Identifier is a global field that is imported with the Library Prep Validation v2.3.0 and NovaSeq X Series Sequencing v1.0 workflows. Illumina Universal Sample Identifier is a text field that is reserved for Clarity LIMS Product Analytics support. The field is optional and not required for this integration.

Terminology

As of Clarity LIMS v5, the term user-defined field (UDF) has been replaced with custom field in the user interface. However, the API resource is still called UDF.

There are two types of custom fields:

- Master step fields—Configured on master steps. Master step fields only apply to the following:
  - The master step on which the fields are configured.
  - The steps derived from those master steps.
- Global fields—Configured on entities (eg, submitted sample, derived sample, measurement, etc.). Global fields apply to the entire Clarity LIMS system.

Step 1: Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.0)

The Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.0) step uses ACTs to configure secondary analysis for samples. Each ACT contains details related to the secondary analysis (eg, index adapter kit to use, reference genome setting, and so on). You can select an ACT from a list and assign samples to it. A planned run can have multiple ACTs and this step must be repeated for each ACT that is required for the run. For more information on creating ACTs and assigning samples to them, refer to *NovaSeq X Series Integration v1.0 Validation and Troubleshooting* on page 129.

The following automations are configured on this step:

- Validate Sample Names and Retrieve Analysis Configuration Template List and Register Step Started
- Retrieve ACT Information
- Validate Reagent Labels and Apply Selected ACT to Samples and Set Next Step
- Register Step Completed

Validate Sample Names and Retrieve Analysis Configuration Template List and Register Step Started Automation

Automatically triggered on entry to the step, this automation completes the following actions:

- Checks that submitted sample names contain only alphanumeric, dash, and underscore characters.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
script:evaluateDynamicExpression -exp 'submittedSamples.each { sample -> if (
!sample.name.matches( ::[A-Za-z0-9_-]+:: ) ) { fail ( ::Submitted sample :: +
sample.name + :: should only have alphanumeric, dash, and underscore characters.
Please rename and try again.:: ) } }' -log {compoundOutputFileLuid1}
```

This script is required for the NovaSeq X Series Sequencing v1.0 workflow.

- Retrieves the list of ACTs that were created in BaseSpace Sequence Hub.

```
/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/novaseqxserie
s-automation.jar script:retrieve_act_list -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1}
```

This script is required for the NovaSeq X Series Sequencing v1.0 workflow.

- Registers the start time of the step by publishing messages to Clarity LIMS Product Analytics through the LINK service.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s
'com/illumina/upa/scripts/common/step_started.groovy'
```

This automation is used only for Clarity LIMS Product Analytics support.

Retrieve ACT Information Automation

Automatically triggered when you select Retrieve ACT Information on the Record Details screen, this automation completes the following actions:

- Retrieves the details of the selected ACT and populates the step custom fields (eg, Analysis Version, Library Prep Kit, and so on).
- Saves the details into a file that is available to download. This file is stored in the Analysis Configuration Metadata file placeholder.

```
/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/novaseqxserie
s-automation.jar script:retrieve_act_info -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} -a '{udf:Analysis Configuration
Template}'
```

Validate Reagent Labels and Apply Selected ACT to Samples and Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation completes the following actions:

- Checks that the molarity of all samples is specified.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
script:evaluateDynamicExpression -t false -h false -exp 'if (!input.hasValue
(::Molarity (nM)::)) { fail(::The Molarity cannot be empty.::) ; } else if
(input.::Molarity (nM):: <= 0) { fail(::The Molarity cannot be negative or
zero.::) ; }' -log {compoundOutputFileLuid1}
```

- Checks that all samples are indexed and for invalid indexes. The indexes applied are the same as the selected ACT.

```
/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/novaseqxserie
s-automation.jar script:validate_indexes -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} -a '{udf:Analysis Configuration
Template}'
```

- Assigns the selected ACT to all samples in the step.

```
/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/novaseqxserie
s-automation.jar script:assign_act -i {stepURI:v2} -u {username} -p {password} -
a '{udf:Analysis Configuration Template}' -l {compoundOutputFileLuid1}
```

- Sets the next step for samples to ADVANCE, which advances them to the next step in the protocol (Make Bulk Pool (NovaSeq X Series Sequencing v1.0)).

```
nextStep = ::ADVANCE:
```

Register Step Completed Automation

Automatically triggered on exit of the step, this automation registers completion time of the step by publishing messages to Clarity LIMS Product Analytics through the LINK service.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s
'com/illumina/upa/scripts/common/step_completed.groovy'
```

This automation is used only for Clarity LIMS Product Analytics support.

Master Step Fields

There are seven fields defined on the Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.0) step. The following table lists field configuration details.

Table 29 Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.0) Master Step Field Configuration

Field Name	Field Type	Options
Analysis Configuration Template	Text Dropdown	Required Field
Application	Text	Read Only
Application Version	Text	Read Only
Index Adapter Kit	Text	Read Only
Library Prep Kit	Text	Read Only
Reference Genome	Text	Read Only
Secondary Analysis Mode	Text	Read Only

Global Fields

The following table lists the global custom fields that are configured to display on the Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.0) step.

Table 30 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
Molarity (nM)	Numeric		Decimal places displayed = 2
ACT Name	Text	Read Only	

User Interaction

For more information, refer to the Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.0) step section in *NovaSeq X Series Integration v1.0 Validation and Troubleshooting* on page 129.

Step 2: Make Bulk Pool (NovaSeq X Series Sequencing v1.0)

Samples are pooled in the Make Bulk Pool (NovaSeq X Series Sequencing v1.0) step. You can manually create working pools based on the final loading concentration required.

The following automations are configured on this step:

- Register Step Started
- Validate Analysis Configurations
- Calculate Volumes

- Set Next Step
- Register Pools & Register Step Completed

Register Step Started Automation

Automatically triggered on entry to the step, this automation registers the start time of the step by publishing messages to Clarity LIMS Product Analytics through the LINK service.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_started.groovy'
```

This automation is used only for Clarity LIMS Product Analytics support.

Validate Analysis Configurations Automation

Automatically triggered on exit of the Pooling screen, this automation performs analysis configuration validity and checks each pool for the following characteristics:

- Pooled samples are within the maximum configuration limit.
- Pooled samples have the same type of analysis (eg, Cloud or Local).
- Pooled samples that have the same secondary analysis also have the same analysis version (eg, v3.8.4).
- Pooled samples that have the same secondary analysis and analysis version also have the same analysis settings (eg, Map/Align Output Format = CRAM).

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/novaseqxseries-automation.jar script:validate_physical_logical_configurations -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -m PerPool script:validate_analysis_config -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -m PerPool && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar script:validate_same_analyte_udf_value_for_all_inputs -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -f 'Run Mode' -perPool true
```

Calculate Volumes Automation

Automatically triggered when you select Calculate Volumes on the Record Details screen, this automation completes the following actions:

- Proceeds if the sample has molarity values.

```
if (!input.hasValue(::Molarity (nM)::)) { return; };
```

- Uses Number of Samples in Pool to calculate the volumes needed for the 2 nM intermediate library pools.

```
if (output.hasValue(::Number of Samples in Pool::)) { output.::Number of Samples in Pool:: = output.::Number of Samples in Pool:: + 1; } else { output.::Number of Samples in Pool:: = 1; }' -t true
```

- Uses the Bulk Pool Volume (µl) field and Number of Lanes to Sequence (x100) for the B3 flow cell type to calculate volumes needed for the 2 nM intermediate library pools.
- Copies the Final Loading Concentration (pM) and Flowcell Type from the step inputs to the step outputs.

```
if (step.::Flowcell Type:: == ::B3::) { output.::Bulk Pool Volume (ul):: = 100 * step.::Number of Lanes to Sequence::; }; output.::NovaSeq X Flowcell Type:: = step.::Flowcell Type::; output.::Final Loading Concentration (pM):: = step.::Final Loading Concentration (pM)::;
```

- Calculates the per sample volume required for each library to make a 2 nM intermediate library pool.

```
input.::Per Sample Volume (ul):: = 2 * output.::Bulk Pool Volume (ul):: / input.::Molarity (nM):: / output.::Number of Samples in Pool::;
```

- If PhiX is used for a planned run (eg, a value for % PhiX (2.0 nM) Spike-In is specified), sets the PhiX Volume (µl).

```
if (step.hasValue(::% PhiX (2.0nM) Spike-In::)) { step.::PhiX Volume (ul):: = step.::% PhiX (2.0nM) Spike-In:: / 100 * step.::Final Loading Volume (ul)::; };
```

- Calculates the adjusted per sample volume for the pools.

```
script:calculate_multipool_adjusted_per_sample_volume -t
```

- Uses the Total Sample Volume (µl) to calculate the RSB volume (µl) required to top up the pools that are needed to create the 2 nM intermediate library pools. The RSB volume is saved to the file in the Calculation File placeholder in the Files section.

```
script:evaluateDynamicExpression \
-exp 'if (output.hasValue(::Total Sample Volume (ul)::)) { output.::Total Sample Volume (ul):: = output.::Total Sample Volume (ul):: + input.::Adjusted Per Sample Volume (ul)::; } else { output.::Total Sample Volume (ul):: = input.::Adjusted Per Sample Volume (ul)::; }' -t true \
script:evaluateDynamicExpression \
-exp 'if (output.::Total Sample Volume (ul):: > output.::Bulk Pool Volume (ul)::) { output.::RSB Volume (ul):: = 0 } else { output.::RSB Volume (ul):: = output.::Bulk Pool Volume (ul):: - output.::Total Sample Volume (ul):: }' -t true \
```

- Uses the NovaSeqXSeries_Bulk_Pool1.csv and NovaSeqXSeries_Bulk_Pool2.csv template files to generate a single CSV file. This file contains information about the pools and the samples that they contain. The generated file is stored in the Calculation File placeholder in the Files section.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeqXSeries_Bulk_
Pool1.csv -o 1.csv \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeqXSeries_Bulk_
Pool2.csv -o 2.csv \
&& cat 1.csv 2.csv > {compoundOutputFileLuid0}.csv \
```

- Resets the Number of Samples in Pool, Total Sample Volume (µl), and Bulk Pool Volume (µl) to null before exiting the step.

```
-exp 'output.::Number of Samples in Pool:: = ::::; output.::Total Sample Volume
(ul):: = ::::; output.::Bulk Pool Volume (ul):: = ::::;' -t true
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation completes the following actions:

- Proceeds only if the sample has a molarity value.
- Copies the Run Mode from input to output.
- Sets the next step for samples to ADVANCE, which advances them to the Dilute and Denature (NovaSeq X Series Sequencing v1.0) step.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} -
log {compoundOutputFileLuid1} \
script:evaluateDynamicExpression -exp 'if (!input.hasValue(::Molarity (nM)::)){
return; }; output.::Run Mode:: = input.::Run Mode::; nextStep = ::ADVANCE::;'"
```

Register Pools & Register Step Completed Automation

Automatically triggered on exit of the step, this automation completes the following actions:

- Registers the pool and library information used by the Clarity LIMS workflow. Samples in the pool are assumed to have gone through library prep.
- Registers completion time of the step by publishing messages to Clarity LIMS Product Analytics through the LINK service.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s
'com/illumina/upa/scripts/common/pooling.groovy' && /opt/gls/clarity/bin/java -
jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-
```

```
product-analytics-automation.jar script:executeUPAAutomationScript -i
{stepURI:v2} -u {username} -p {password} -s
'com/illumina/upa/scripts/common/step_completed.groovy'
```

This automation is used only for Clarity LIMS Product Analytics support.

Master Step Fields

There are seven fields defined on the Make Bulk Pool (NovaSeq X Series Sequencing v1.0) step. The following table lists configuration details.

Table 31 Make Bulk Pool (NovaSeq X Series Sequencing v1.0) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
% PhiX (2.5 nM) Spike-In	Numeric		Range = 0–100
Final Loading Concentration (pM)	Numeric Dropdown	Required Field Custom Entries	
Final Loading Volume (µl)	Numeric	Required Field	Default • 200
Flowcell Type	Text	Required Field Read Only	Default • B3
Minimum Per Sample Volume (µl)	Numeric	Required Field	Decimal places displayed = 2 Default • 2
Number of Lanes to Sequence	Numeric	Required Field	Range = 1–10 Decimal places displayed = 0

Field Name	Field Type	Options	Additional Options and Dropdown Items
PhiX Volume (µl)	Numeric	Read Only	Hidden Decimal places displayed = 1 Used for Calculate Volume script when there is PhiX spike-in. Do not remove, rename, or modify this field as it can cause the integration to break.

Global Fields

The following table lists the global custom fields that are configured to display on the Make Bulk Pool (NovaSeq X Series Sequencing v1.0) step.

Table 32 Global Custom Fields Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
Final Loading Concentration (pM)	Numeric Dropdown	Required Field Custom Entries	Decimal places displayed = 0 Presets • 225 • 400
RSB Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
NovaSeq X Flowcell Type	Text	Required Field Read Only	Default • B3

User Interaction

For more information, refer to Run Make Bulk Pool (NovaSeq X Series Sequencing v1.0) step section in *NovaSeq X Series Integration v1.0 Validation and Troubleshooting* on page 129.

Step 3: Dilute and Denature (NovaSeq X Series Sequencing v1.0)

The Dilute and Denature (NovaSeq X Series Sequencing v1.0) step allows you to dilute pooled samples with the addition of RSB.

The following automations are configured on this step:

- Validate Inputs Flowcell Type and Register Step Started
- Calculate Volumes
- Set Next Step
- Register Step Completed

Validate Inputs Flowcell Type and Register Step Started

Automatically triggered on entry to the step, this automation completes the following actions:

- Checks that the specified flow cell type is valid.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
script:evaluateDynamicExpression -h false -exp 'if (!input.hasValue(::NovaSeq X
Flowcell Type::)){fail(::Invalid Flowcell Type custom field value. Please make
sure all input samples have a value assigned for Flowcell Type custom
field.::)}' -log {compoundOutputFileLuid1}
```

  This script is required for the NovaSeq X Series Sequencing v1.0 workflow.

- Registers the start time of the step by publishing messages to Clarity LIMS Product Analytics through the LINK service.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s
'com/illumina/upa/scripts/common/step_started.groovy'
```

  This automation is used only for Clarity LIMS Product Analytics support.

Calculate Volumes Automation

Automatically triggered when you enter the Record Details screen, this automation completes the following actions:

- Checks that the NovaSeq X Flowcell Type is B3 and sets NaOH Volume (µl) and TT2 Volume (µl) field values to 10 and 150.
- Computes BP Aliquot Volume (µl) and RSB Volume (µl) required for the dilution of pools to the required final loading concentration.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} -
log {compoundOutputFileLuid1} \
```

```
script:evaluateDynamicExpression \
-exp 'if (input.::NovaSeq X Flowcell Type:: == ::B3::) { output.::NaOH Volume
(ul):: = 10; output.::TT2 Volume (ul):: = 150; }; output.::BP Aliquot Volume
(ul):: = input.::Final Loading Concentration (pM):: * 200 / (2 * 1000);
output.::RSB Volume (ul):: = 40 - output.::BP Aliquot Volume (ul)::;'
```

- Generates a single CSV file that contains information about the reagents volume required to dilute the working pools. The generated file is stored in the Calculation File placeholder in the Files section.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeqXSeries_Dilute_
Denature_Calculate_Volumes.csv -o {compoundOutputFileLuid0}.csv -q true -
destLIMSID {compoundOutputFileLuid0} -l {compoundOutputFileLuid1}"
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation completes the following actions:

- Copies the Run Mode from input to output.
- Sets the next step for samples to ADVANCE, advancing them to the Load to Library Tube Strip (NovaSeq X Series Sequencing v1.0) step.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
script:evaluateDynamicExpression -t false -h false -exp 'output.::Run Mode:: =
input.::Run Mode::; nextStep = ::ADVANCE::' -log {compoundOutputFileLuid1}
```

Register Step Completed Automation

Automatically triggered on exit of the step, this automation registers the completion time of the step by publishing messages to Clarity LIMS Product Analytics through the LINK service.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s
'com/illumina/upa/scripts/common/step_completed.groovy'
```

This automation is used only for Clarity LIMS Product Analytics support.

Global Fields

The following table lists the global custom fields that are configured to display on the Dilute and Denature (NovaSeq X Series Sequencing v1.0) step.

Table 33 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
BP Aliquot Volume (µl)	Numeric	Read Only	Decimal places displayed = 0
NaOH Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
RSB Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
TT2 Volume (µl)	Numeric	Read Only	Decimal places displayed = 2

User Interaction

For more information, refer to the Dilute and Denature (NovaSeq X Series Sequencing v1.0) step section in *NovaSeq X Series Integration v1.0 Validation and Troubleshooting* on page 129.

Step 4: Load to Library Tube Strip (NovaSeq X Series Sequencing v1.0)

The Load to Library Tube Strip (NovaSeq X Series Sequencing v1.0) step allows you to scan the library tube barcode into Clarity LIMS. Then, you can place the working pools into the library tube strip used in the NovaSeq X Series run. This step also does the following actions:

- Validates the run setup and analysis information.
- Generates the sample sheet file.
- Creates a planned run on ICA, depending on the selected run mode.

The following automations are configured on this step:

- Validate Input Count and Validate Analysis Configurations and Register Step Started
- Validate Library Tube Strip Barcode
- Validate Run Setup and Create Planned Run
- Set Next Step
- Register Step Completed

Validate Input Count and Validate Analysis Configurations and Register Step Started

Automatically triggered on entry to the step, this automation completes the following actions:

- Checks that the number of input pools is the same as the number of tubes in the library tube strip.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
script:validateSampleCount -min 8 -max 8
```

  This script is required for the NovaSeq X Series Sequencing v1.0 workflow.

- Performs basic checks on the secondary analysis configuration of the samples in the same planned run. The following checks are included in this script:
  - Secondary analysis configuration of samples in a planned run is within maximum configuration limit.
  - Samples in a planned run have the same type of analysis (eg, Cloud or Local).
  - Samples in a planned run that have the same secondary analysis and analysis version (eg, Illumina DRAGEN Enrichment) also have the same analysis settings (eg, Map/Align Output Format = CRAM).
  - Samples in a planned run that have the same secondary analysis have the same analysis version (eg, v3.8.4).

```
/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/novaseqxser
ies-automation.jar script:validate_physical_logical_configurations -i
{stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -m
PerRun script:validate_analysis_config -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} -m PerRun &&
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar script:validate_same_analyte_udf_value_for_
all_inputs -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} -f 'Run Mode' -perPool false
```

  This script is required for the NovaSeq X Series Sequencing v1.0 workflow.

- Registers the start time of the step by publishing messages to Clarity LIMS Product Analytics through the LINK service.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s
'com/illumina/upa/scripts/common/step_started.groovy'
```

  This script is used only for Clarity LIMS Product Analytics support.

Validate Library Tube Strip Barcode Automation

Automatically triggered on the Placement screen, this automation validates the library tube barcode to make sure that it conforms to the barcode mask LC[0-9]{7}-LC1.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
script:validate_output_containers -l {compoundOutputFileLuid1} -r 'Library 8-
tube Strip:LC[0-9]{7}-LC1' -max 1
```

Validate Run Setup and Create Planned Run Automation

Automatically triggered after a selection on the Record Details screen, this automation completes the following actions:

- Validates the parameters entered on the Record Details screen. These parameters are used to set up the run, generate the sample sheet file, and create the planned run in ICA.
  - Run Name can only contain alphanumeric, dash, underscore, or period characters. Do not use spaces.

    ```
    if (!step.::Run Name::.matches(::[a-zA-Z0-9-_]+[a-zA-Z0-9-_. ]*::)) { fail
    (::Run Name contains prohibited characters. Please check to make sure Run
    Name only contains alphanumeric characters, spaces, dashes and underscores.
    Run Name must start with alphanumeric, a dash or an underscore::); }
    ```

  - Run Name cannot exceed 255 characters.

    ```
    if (step.::Run Name::.length() > 255) { fail(::Run Name shall not exceed 255
    characters.::); };
    ```

- Checks the Index 1 Cycles and Index 2 Cycles field values. If Index 2 Cycles is greater than 0, the Index 1 Cycles value must be greater than 0 or an error can occur.

```
if (step.::Index 2 Cycles:: > 0 && step.::Index 1 Cycles:: == 0) { fail(::Index
1 Cycles cannot be zero if Index 2 Cycles is non-zero::); };
```

- Generates the sample sheet. If you are in Cloud run mode, the automation creates the planned run in ICA. The sample sheet is attached to the step.

```
/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/novaseqxserie
s-automation.jar script:generate_sample_sheet -i {stepURI:v2} -u {username} -p
{password} -c {compoundOutputFileLuid0} -l {compoundOutputFileLuid1}
```

There is a backend validation of the sample sheet content in ICA. This validation makes sure that the sample sheet is valid to set up a Local mode run and that it is ready for use with DRAGEN applications.

For more information on sample name character restrictions, refer to *NovaSeq X Series Integration v1.0 Validation and Troubleshooting* on page 129.

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation completes the following actions:

- Copies the Run Mode from input to output.
- Sets the next step for samples to ADVANCE, advancing them to the AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0) step.

```
output.::Run Mode:: = input.::Run Mode::; nextStep = ::ADVANCE::
```

Register Step Completed Automation

Automatically triggered on exit of the step, this automation registers the completion time of the step by publishing messages to Clarity LIMS Product Analytics through the LINK service.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s 'com/illumina/upa/scripts/common/step_completed.groovy'
```

This automation is used only for Clarity LIMS Product Analytics support.

Master Step Fields

The following table shows the master step fields that are configured on the Load to Library Tube Strip (NovaSeq X Series Sequencing v1.0) step. These fields are required for sample sheet generation and planned run creation in ICA.

Table 34 Clarity LIMS Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Cloud Run ID	Text	Read Only	Hidden Used for creating a planned run in ICA. Do not remove, rename, or modify this field as it can cause the integration to break.

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Field Name	Field Type	Options	Additional Options and Dropdown Items
Index 1 Cycles	Numeric Dropdown	Required Field Custom Entries	Range = 0–20 Presets • 0 • 6 • 8
Index 2 Cycles	Numeric Dropdown	Required Field Custom Entries	Range = 0–20 Presets • 0 • 6 • 8
Output Folder	Text		
Read 1 Cycles	Numeric Dropdown	Required Field Custom Entries	Range = 1–251 Presets • 51 • 101 • 151
Read 2 Cycles	Numeric Dropdown	Required Field Custom Entries	Range = 0–251 Presets • 51 • 101 • 151
Run Mode	Text Dropdown	Required Field	Presets • Local • Cloud
Run Name	Text	Required Field	

User Interaction

For more information, refer to the Load to Library Tube Strip (NovaSeq X Series Sequencing v1.0) step section in *NovaSeq X Series Integration v1.0 Validation and Troubleshooting* on page 129.

Step 5: AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0)

The AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0) step is fully automated. The integration starts the step, adds samples to the Ice Bucket, and completes the step. Data from the run is parsed back to Clarity LIMS. In this step, pooled samples in the reagent cartridge are sequenced on the NovaSeq X Series instrument.

The following automations are configured on this step:

- Register Step Started & Register NovaSeq X Run & Register NovaSeq X Run Association & Register NovaSeq X RunStarted
- Register NovaSeq X RunCompleted & Register Step Completed

Register Step Started & Register NovaSeq X Run & Register NovaSeq X Run Association & Register NovaSeq X RunStarted Automation

Automatically triggered at the beginning of the step, this automation completes the following actions:

- Registers the start time of the step.
- Registers the NovaSeq X Series sequencing run configuration.
- Registers the association of the sequencing run with the samples by linking the Instrument Run ID with the sample ID.
- Registers the time that the sequencing run started.

This automation completes these actions by publishing messages to Clarity LIMS Product Analytics through the LINK service.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s
'com/illumina/upa/scripts/common/step_started.groovy' &&
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s
'com/illumina/upa/scripts/novaseqxseries/register_novaseqxseries_run.groovy' &&
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s
'com/illumina/upa/scripts/novaseqxseries/novaseqxseries_associate_seqrun.groovy'
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s
'com/illumina/upa/scripts/novaseqxseries/novaseqxseries_seqrun_started.groovy'
```

The automation is used only for Clarity LIMS Product Analytics support.

Register NovaSeq X RunCompleted & Register Step Completed Automation

Automatically triggered on exit of the step, this automation completes the following actions:

- Registers the sequencing run completion time, run status, and run metrics. This script requires the Cloud Run ID custom field from the Load to Library Tube Strip (NovaSeq X Series Sequencing v1.0) step.

- Registers the completion time of the step.

This automation completes these actions by publishing messages to Clarity LIMS Product Analytics through the LINK service.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s 'com/illumina/upa/scripts/novaseqxseries/novaseqxseries_seqrun_completed.groovy' && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -s 'com/illumina/upa/scripts/common/step_completed.groovy'
```

This automation is used only for Clarity LIMS Product Analytics support.

Master Step Fields

The following tables show the master step fields that are configured on the AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0) step.

Table 35 Clarity LIMS Master Step Field Information

Field Name	Source File	Corresponding Field in Source File	Additional Information
Current Cycle	`RunCompletionStatus.xml`	CompletedReads	Visible on Record Details screen. Calculated based on the CompletedReads field.
Current Read	`RunCompletionStatus.xml` `RunParameters.xml`	CompletedReads PlannedReads	Visible on Record Details screen. Calculated based on the CompletedReads field in `RunCompletionStatus.xml` and PlannedReads field in `RunParameters.xml`.
Flow Cell Expiration Date	`RunParameters.xml`	ExpirationDate	Visible on Record Details screen. Corresponds to the value in the ExpirationDate field under the ConsumableInfo field for FlowCell Type.

Field Name	Source File	Corresponding Field in Source File	Additional Information
Flow Cell ID	`RunParameters.xml`	SerialNumber	Visible on Record Details screen. Corresponds to the value in the SerialNumber field under the ConsumableInfo field for FlowCell Type.
Flow Cell Lot Number	`RunParameters.xml`	LotNumber	Visible on Record Details screen. Corresponds to the value in the LotNumber field under the ConsumableInfo field for FlowCell Type.
Flow Cell Part Number	`RunParameters.xml`	PartNumber	Visible on Record Details screen. Corresponds to the value of the PartNumber field under the ConsumableInfo field for FlowCell Type.
Flow Cell Side	`RunParameters.xml`	Side	Visible on Record Details screen.
Flow Cell Type	`RunParameters.xml`	FlowCellType	Visible on Record Details screen.
Instrument Control Software Version	`RunParameters.xml`	SystemSuiteVersion	Visible on Record Details screen.
Instrument ID	`RunParameters.xml`	InstrumentSerialNumber	Visible on Record Details screen.
Instrument Type	`RunParameters.xml`	InstrumentType	Visible on Record Details screen.
Output Folder	`RunParameters.xml`	OutputFolder	Visible on Record Details screen.

Field Name	Source File	Corresponding Field in Source File	Additional Information
Library Tube Barcode	`RunParameters.xml`	SerialNumber	Visible on Record Details screen. Corresponds to the value of the SerialNumber field under the ConsumableInfo field for SampleTube Type.
Library Tube Lot Number	`RunParameters.xml`	LotNumber	Hidden Do not remove, rename, or modify this field as it can cause the integration to break. Corresponds to the value of the LotNumber field under the ConsumableInfo field for SampleTube Type.
Run End Time	`RunCompletionStatus.xml`	RunEndTime	Hidden Do not remove, rename, or modify this field as it can cause the integration to break.
Run Name	`RunParameters.xml`	ExperimentName	Visible on Record Details screen.

Table 36 Other Clarity LIMS Master Step Fields

Field Name	Value	Additional Information
Run Status	Presets • RunStarted • RunCompletedSuccessfully • RunAbortedByUser • RunErroredOut Set by the SIS integration service.	Visible on Record Details screen.

Field Name	Value	Additional Information
Sequencing Log	Set by the SIS integration service during the sequencing run.	Visible on Record Details screen.
BaseSpace Run ID	Received from the SQS message in the SIS integration service.	Hidden Do not remove, rename, or modify this field as it can cause the integration to break.
ICA Project ID	Received from the SQS message in the SIS integration service.	Hidden Do not remove, rename, or modify this field as it can cause the integration to break.
Instrument Run ID	Received from the SQS message in the SIS integration service.	Hidden Do not remove, rename, or modify this field as it can cause the integration to break.
Run Start Time	Received from the SQS message in the SIS integration service.	Hidden Do not remove, rename, or modify this field as it can cause the integration to break.

Global Fields

The following global custom fields are used to capture the run metrics in Clarity LIMS:

- % Bases >=Q30 R1
- % Bases >=Q30 R2
- % Error Rate R1
- % Error Rate R2
- Yield (Gb) R1
- Yield (Gb) R2
- Reads PF R1
- Reads PF R2

- %PF R1
- %PF R2
- % Aligned R1
- % Aligned R2
- % Phasing R1
- % Phasing R2
- % PrePhasing R1
- % PrePhasing R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- Cluster Density R1
- Cluster Density R2

At the end of the step, the pools of samples are automatically removed from the step. The step completes automatically when Run Status is RunCompletedSuccessfully.

How the Integration Works

The following information summarizes how the NovaSeq X Series integration works.

- After the Validate Run Setup and Create Planned Run automation is triggered on the Load to Library Tube Strip (NovaSeq X Series Sequencing v1.0) step, the run parameters entered in the Run Details screen are sent to ICA. The analysis configuration parameters from the selected ACTs are also sent to ICA. ICA validates the run and analysis configuration. If the validation fails, ICA sends an error message to Clarity LIMS. If the validation passes, ICA generates the sample sheet and sends it back to Clarity LIMS. ICA also creates the planned run based on the selected run mode (eg, Local or Cloud).
  - For the Local run mode, the sample sheet is generated and stored in Clarity LIMS. This sample sheet contains the run and analysis configuration information required to start the run on the NovaSeq X Series instrument.
  - For the Cloud run mode, the planned run is created in ICA. The planned run contains the run and analysis configuration information required to start the run on the instrument. The sample sheet is generated and stored in Clarity LIMS for reference purposes.
- When the sequencing run starts on the instrument, the NovaSeq X Series Control Software notifies BaseSpace Sequence Hub. BaseSpace Sequence Hub monitors the run and uses the Stratus Integration Service (SIS) to notify Clarity LIMS. The events are processed and the integration service retrieves the run information from BaseSpace Sequence Hub. This information is used to populate the custom fields in the AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0) step.

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- Other run events follow the same information flow. When sequencing is complete, the control software uploads the sequencing run data (primary metrics) and associated files to ICA. Then, Clarity LIMS retrieves the primary metrics and uses them to populate the fields in the Sample Details table (eg, % Error Rate R1). The custom fields (eg, Run Status, Current Read, and so on) on the AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0) step are updated using the run related information. If the sequencing run is successfully completed, the step automatically completes.
- The analysis results are not tracked. You must log in to BaseSpace Sequence Hub to retrieve the cloud analysis results. For local analysis, the secondary analysis results are found in the external storage configured in Illumina Run Manager. You can find this storage information under External Storage and Analysis Results.

NovaSeq X Series Run

The following steps outline the sequence of events that occurs when a flow cell is loaded onto the NovaSeq X Series instrument.

Run Mode Configuration

1. In the NovaSeq X Series Control Software, log in to the instrument using an account that has permissions for configuring settings.
2. From the menu, select Settings.
3. From Settings, select Cloud settings.
4. Under Run Settings, select the checkbox next to Cloud run storage.
5. For Hosting location, select the applicable location from the drop-down options.
6. [Optional] For Private domain name, enter the domain name according to the workgroup that was configured with the NovaSeq X Series integration package.
7. Select Save.

Start a Sequencing Run in Local Run or Cloud Modes

1. You can start the sequencing run in BaseSpace Sequence Hub for both the Local and BaseSpace (Cloud) modes. Make sure that you log in using the account that was used during the NovaSeq X Series integration package setup. For more information, refer to the BaseSpace Sequence Hub Online Help at help.basespace.illumina.com.

Components Installed

The following sections describe the components (files, properties, reagent categories / label groups, reagent kits, and containers) that are installed by default as part of this integration.

Reagent Kits

- Buffer Cartridge

- Lyophilization Cartridge
- NaOH
- Reagent Cartridge
- Resuspension Buffer (RSB)
- TT2

Container Types

- Library 8-Tube Strip
- Tube

This integration supports the library 8-tube strip with the barcode in the LC[0-9]{7}-LC1 format (eg, LC1234567-LC1).

Control Types

- PhiX v3

Rules and Constraints

- The workflow configuration contains several validation checks. To make sure that the calculations work properly, it is important that you do not disable any of this validation logic. The validation checks determine the following information:
  - Which samples, and how many, can enter each step together.
  - Which samples, and how many, can be pooled together.
- All submitted samples must have an associated secondary analysis that is configured using the analysis configuration template (ACT). The ACT must be configured on BaseSpace Sequence Hub before starting the Assign Analysis Configuration Template step.
- The library tube strip barcode must be unique. There must not be multiple library tube strip containers with the same name in the system.
- Reagent labels, or indexes, must be unique.
- One library pool can only contain one library or control with no label/index.
- The AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0) step must not be manually started or completed. This step is fully automated and the sequencing service does not update samples correctly if they have been manually started.
- For the automated run to start successfully, you must select Validate Run Setup and Create Planned Run in the Load to Library Tube step.

NovaSeq X Series Integration v1.0 Validation and Troubleshooting

This section explains how to validate the installation of the Illumina NovaSeq X Series Integration Package v1.0.

The validation process involves the following actions:

- Running samples through the Library Prep Validation v2.3.0 workflow.
  - The workflow contains a single-step protocol that models the library prep required to produce libraries tagged with index sequences. At the end of the step, these libraries are routed to NovaSeq X Series Sequencing v1.0 workflow.
- Running libraries through the NovaSeq X Series Sequencing v1.0 workflow validates the following items:
  - Successful sequential step advancement of samples through the steps of the workflow.
  - Automatic validation of run setup information before sample sheet generation or planned run creation.
  - Automated sample sheet generation. This sample sheet is used to start the sequencing run on the NovaSeq X Series Control Software through the Local run mode.
  - Automated creation of a planned run in Illumina Connected Analytics (ICA). The control software retrieves the planned run. The run is started through the Cloud run mode.
  - Automated tracking of the NovaSeq X Series sequencing run and parsing of run statistics into Clarity LIMS.

The validation steps assume that the following conditions have been met:

- NovaSeq X Series Integration Package v1.0 is installed and you have imported the default Clarity LIMS configuration.
- Analysis configuration templates (ACTs) are created in BaseSpace Sequence Hub for your run. Make sure that the index adapter kit label group is created in Clarity LIMS before selecting it in the ACT. This same label group is used in the Run Library Prep Validation v2.3.0 step. For more information on creating a reagent label group, refer to *Add and Configure Labels and Label Groups* in the Clarity LIMS Core Documentation. For the adapter sequences for your library prep kits, refer to *Illumina Adapter Sequences*.

Analysis Configuration Template Creation in BaseSpace Sequence Hub

You must create the analysis configuration templates (ACTs) that are required for configuring secondary analysis in the NovaSeq X Series Sequencing v1.0 workflows. Create ACTs in BaseSpace Sequence Hub. For instructions, refer to the BaseSpace Sequence Hub online help.

Activate Workflow, Create Project, Add and Assign Samples

The following steps set up Clarity LIMS in preparation for running samples through the Library Prep Validation v2.3.0 and NovaSeq X Series Sequencing v1.0 workflows.

1. On the Configuration tab, under Workflows, activate both the Library Prep Validation v2.3.0 and NovaSeq X Series Sequencing v1.0 workflows.
2. On the Projects and Samples screen, create a project and add samples to it.

   Only use alphanumeric, dash, or underscore characters in the submitted sample names. Invalid characters can cause a sample sheet validation failure in the Load to Library Tube Strip step.

3. Assign the samples to the Library Prep Validation workflow.

Library Prep Protocol: Library Prep Validation v2.3.0

This single-step protocol models the library prep required to produce libraries tagged with index sequences that are ready for the NovaSeq X Series Sequencing v1.0 workflow.

Run Library Prep Validation v2.3.0

1. In Lab View, locate the Library Prep Validation v2.3.0 protocol.
   Samples are queued for the Library Prep Validation v2.3.0 step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, under Label Options, select the label groups that you want to use.
   The label group, or index adapter kit, selected must be the same as the Index Adapter Kit selected in the ACT that is being used.
4. Select Begin Work.
5. On the Placement screen, complete the following actions:
   a. In the Placed Samples area on the right, in the Container field, scan or enter the barcode of the destination container.
   b. Select the samples from the Samples to be Placed area on the left, and drag them to the container wells on the right.
   c. Select Add Labels.
6. On the Add Labels screen, the Labels to Add list contains labels from the label group selected in step 3 (eg, TruSeq HT Adapters v2 (D7-D5)). TruSeq HT Adapters v2 (D7-D5) is the only label group configured in the integration and is used by default in this step.
   a. Select labels on the left, and drag them to the container on the right to assign them to placed samples.
   b. Select Record Details.
7. On the Record Details screen, complete the following actions:

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a. In the Sample Details table, enter values for Molarity (nM). Type values individually or copy and paste to enter multiple values from a spreadsheet.

> The Molarity (nM) values are used in the Calculate Volume automation script in the Make Bulk Pool (NovaSeq X Series Sequencing v1.0) step. If these values are not entered, the automation script does not work properly.

b. The Sequencing Instrument column indicates the instrument to be used for the sequencing run. Select NovaSeq X Series.

c. Populate the fields in the Sequencing Instrument column using one of the following methods:

- To populate fields individually for each sample, select from the drop-down list in each row.
- To populate all fields at one time, copy and paste from a spreadsheet.
- Alternatively, select inside the table and press Ctrl + A to select all rows. At the top of the table, select Sequencing Instrument and then select NovaSeq X Series from the adjacent drop-down list. Select Apply.

> If working with many samples, copy and paste to insert multiple values from a spreadsheet as follows.

a. Select Copy Samples to copy the sample information from Clarity LIMS to the Clipboard.

b. Open a blank Excel document and paste the contents of the clipboard into the spreadsheet.

c. In the spreadsheet, enter the Molarity (nM) and Sequencing Instrument values. Select the entire spreadsheet including the headers (Ctrl + A), and copy (Ctrl + C) the information to the Clipboard.

d. Within Clarity LIMS, select the Paste Samples button. Paste the contents of the clipboard (Ctrl + V) into the Update Table window and then select Update to paste the contents into the Sample Details table.

8. Select Next Steps.
   This selection triggers the Next Steps automation, which sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step.
9. On the Assign Next Steps screen in the Samples table, the Next Step value for all samples is prepopulated with Remove from workflow.

   > Do not change this value. If Next Step is not set to Remove from workflow, the routing script does not route samples correctly.

10. Select Finish Step.
    On exit from the step, the Routing Script automation is triggered. This automation assigns samples to the first step of the NovaSeq X Series Sequencing v1.0 workflow, Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.0).

Protocol: NovaSeq X Series Sequencing v1.0

The NovaSeq X Series Sequencing v1.0 protocol consists of the following steps:

- Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.0)
- Make Bulk Pool (NovaSeq X Series Sequencing v1.0)
- Dilute and Denature (NovaSeq X Series Sequencing v1.0)
- Load To Library Tube Strip (NovaSeq X Series Sequencing v1.0)
- AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0)

Each step contains a script to register the start time (upon step entry) and the time that the step is completed (upon step exit). This information is published to Clarity LIMS Product Analytics through the Link service. These scripts are used only for Clarity LIMS Product Analytics support and can be incorporated as part of an automation that performs other functions.

Step 1: Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.0)

1. In Lab View, locate the NovaSeq X Series Sequencing v1.0 protocol. The samples are queued for the Assign Analysis Configuration Template (NovaSeq X Series Sequencing v1.0) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work to start the Validate Sample Names and Retrieve Analysis Configuration Template List and Register Step Started automation.
   The automation checks that the sample names do not use restricted characters and are within character limits and retrieves the list of ACTs that you can select. The automation also registers the start time of the step by publishing messages to Clarity LIMS Product Analytics through the LINK service. The part of the script for start time registration is used only for Clarity LIMS Product Analytics support.
4. Select the applicable ACT to assign to the samples. The index adapter kit specified by the ACT must correspond with the label group used in *Library Prep Protocol: Library Prep Validation v2.3.0* on page 130.
5. [Optional] Under Step Details, select Retrieve ACT Information to trigger the Retrieve ACT Information automation.
   This automation retrieves ACT information (eg, Library Prep Kit, Index Adapter Kit, Reference Genome, and so on) and populates the fields in Clarity LIMS. These details are saved to the `ACTMetadata.csv` file that you can download. If you are not sure of the analysis configuration before starting the sequencing and analysis run, refer to the details in the file.
6. Select Next Steps to assign the ACT to the samples.

This action triggers the Validate Reagent Labels and Apply Selected ACT to Samples and Set Next Step automation. This automation validates that the indexes applied to the libraries are valid for the selected ACT and assigns samples to it. All samples added to the Ice Bucket in step 2 are assigned to this ACT.

7. Select Finish Step.

To use multiple ACTs in a single run, repeat steps 2–7 for each ACT. For step 2, only add samples that are being assigned to that ACT into the Ice Bucket.

Step 2: Make Bulk Pool (NovaSeq X Series Sequencing v1.0)

1. In Lab View, locate the NovaSeq X Series Sequencing v1.0 protocol. The samples are queued for the Make Bulk Pool (NovaSeq X Series Sequencing v1.0) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
   You can also add control samples by selecting them from the Available control samples drop-down list and selecting Add.
3. On the Ice Bucket screen, select Begin Work.
4. Create a pool of samples as follows.
   a. On the Pooling screen, create a pool by dragging samples into the Pool Creator.
      If a control sample was added, it appears under the Sample List and can be pulled into the pool that needs the control.
   b. Enter a name for your pool or accept the default name (Pool #1).
   c. [Optional] If multiple pools are required, select the plus sign (+) next to Pool Creator to create a pool.
   d. [Optional] To remove a pool, select the X in the top right corner of the pool.
   e. Select Record Details to trigger the Validate Analysis Configurations automation.
      This automation performs the following checks on the analysis configuration for each pool:
      - Pooled samples are within the maximum configuration limit.
      - Pooled samples have the sample type of analysis (Cloud or Local).
      - Pooled samples that have the same secondary analysis also have the same analysis version.
      - Pooled samples that have the same secondary analysis also have the same analysis settings.
5. On the Record Details screen, navigate to the Reagent Lot Tracking section to track the Universal Buffer (UNB) lot information used in the step.
6. [Optional] Create a new lot. For more information, refer to *Add and Configure Reagent Kits and Lots* in the Clarity LIMS LIMS Core Functionality documentation.
7. In the Step Details area, complete the following required fields:

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- Number of Lanes to Sequence—Used in volume calculations, to make sure that the volumes are sufficient for the number of times the pool is sequenced. This field is applied to all pools in the step. If a pool requires a different value for this field, then repeat the Make Bulk Pool (NovaSeq X Series Sequencing v1.0) step.
- Final Loading Concentration (pM)—The final loading concentration of the pool in the flow cell. This field value is typically dependent on the library type.
- Final Loading Volume (µl)—The final loading volume of the pool in the flow cell. This field is prepopulated with the configured default value (200 µl), but can be edited if more volume is required.
- Minimum Per Sample Volume (µl)—The minimum volume for each sample. This field is prepopulated with the configured default value (2 µl) and can be edited. If the per sample volume is below the value set in this field, the Calculate Volumes script applies a rounding factor to affected samples so that the volume reaches the minimum volume.

8. Select Calculate Volumes to trigger the Calculate Volumes automation.
   This automation calculates the volumes required for each library to form a pool that has the concentration and volume specified in the Step Details field. This automation is applied to control samples when they are indexed and molarity is specified. It also generates the Calculation File in a CSV format and attaches it to the step. You can select the file name to download it.
9. In the Sample Details table, select the pool next to the sample name to view details on the pool composition.
10. Select Next Steps to trigger the Set Next Step automation.
    This automation sets the next step for samples to ADVANCE, which moves them to the Dilute and Denature (NovaSeq X Series Sequencing v1.0) step.
11. On the Assign Next Steps screen, make sure that the samples are set to the Dilute and Denature (NovaSeq X Series Sequencing v1.0) step.
12. Select Finish Step.

Step 3: Dilute and Denature (NovaSeq X Series Sequencing v1.0)

1. In Lab View, locate the NovaSeq X Series Sequencing v1.0 protocol. The pool of samples queued for the Dilute and Denature (NovaSeq X Series Sequencing v1.0) displays.
2. Add the pool to the Ice Bucket and select View Ice Bucket.
3. [Optional] On the Ice Bucket screen, set the number of derivatives to create (placed into the flow cell lanes) and select Begin Work.
   - On entry to the step, the Validate Inputs Flowcell Type and Register Step Started automation is triggered. This automation makes sure that the configured flow cell type is valid. The automation also registers the start time of the step by publishing messages to Clarity LIMS Product Analytics through the LINK service. The portion of the script used for start time registration is used only for Clarity LIMS Product Analytics support.

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- On entry to the Record Details screen, the Calculate Volumes automation is triggered.
  This automation sets the following values:
  - BP Aliquot Volume (µl)
  - RSB Volume (µl)
  - NaOH Volume (µl)
  - TT2 Volume (µl)
  - The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains information about the volume of RSB, NaOH, and TT2 to add per working pool.

4. On the Record Details screen, the Reagent Lot Tracking section tracks the NaOH, Resuspension Buffer, and TT2 reagents used in the step. To add and activate reagent lots, do as follows.
   a. In a new browser tab, open an additional instance of Clarity LIMS.
   b. Navigate to the Consumables configuration screen.
   c. Select one of the reagent kits previously listed (eg, NaOH) and select New Lot.
   d. Enter the lot details.
   e. Select Save.
   f. Set the Status of Reagent Lot toggle switch to Active.
   g. Repeat this process to add and activate the remaining reagent lots.
   h. Return to your original working browser tab and refresh the page.
5. On the Record Details screen, perform the following actions:
   a. In the Reagent Lot Tracking section, select from the active lots displayed in each drop-down list.
   b. In the Sample Details table, make sure that the BP aliquot, RSB, NaOH, and TT2 reagent volume values are populated. The script sets these values and they cannot be edited.
   c. [Optional] In the Sample Details table, select the pool icon to view details of the working pool composition.
      The working pool number is appended to the bulk pool name so that you can identify which working pools are derived from the same bulk pool.
6. In the Files area, select the Calculation File (CSV) to open it and view details on the RSB, NaOH, and TT2 volumes to add per working pool.
7. Select Next Steps.
   On the Assign Next Steps screen, the next step is already set to Load to Library Tube Strip (NovaSeq X Series Sequencing v1.0).
8. Select Finish Step.

Step 4: Load to Library Tube Strip (NovaSeq X Series Sequencing v1.0)

1. In Lab View, locate the NovaSeq X Series Sequencing v1.0 protocol. The pool of samples are queued for the Load to Library Tube Strip (NovaSeq X Series Sequencing v1.0) step.

2. Add the pools to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work to trigger the Validate Analysis Configurations and Register Step Started automation.
   This automation performs the following basic checks on the analysis configuration for each pool:
   - Pooled samples are within the maximum configuration limit.
   - Pooled samples have the sample type of analysis (Cloud or Local).
   - Pooled samples that have the same secondary analysis alsohave the same analysis version.
   - Pooled samples that have the same secondary analysis also have the same analysis settings.

   The automation also registers the start time of the step by publishing messages to Clarity LIMS Product Analytics through the LINK service. The portion of the script used for start time registration is used only for Clarity LIMS Product Analytics support.
4. On the Placement screen, do as follows.
   a. Drag the pool into the Library 8-tube Strip in the Placed Samples area on the right.
   b. Scan or type the barcode of the Library 8-tube Strip into the Library 8-tube Strip container name field.
   c. Select Record Details.

   After exiting the Placement screen, the Validate Library Strip Tube Barcode automation makes sure that the library tube strip barcode conforms to the barcode mask LC[0-9]{7}-LC1.
5. The fields displayed on the Record Details screen are used to create the planned run and generate the sample sheet.
   The analysis related information for the planned run is from the ACT associated with the samples and no further analysis configuration is required. Refer to the following table for details.

Table 37 Fields Displayed on Record Details Screen of Load to Library Tube Strip (NovaSeq X Series Sequencing v1.0) Step

Field	Value
Run Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. No spaces.
Run Mode	The selected Run Mode must correspond to the ACT type. Presets • Local[1] • Cloud[2]
Read 1 Cycles	Presets • 151 • 101 • 51 • Type a custom value

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Field	Value
Read 2 Cycles	Presets • 151 • 101 • 51 • Type a custom value
Index 1 Cycles	Presets • 0 • 6 • 8 • Type a custom value[3]
Index 2 Cycles	Presets • 0 • 6 • 8 • Type a custom value[3]

[1] The Local run mode generates the sample sheet. The Run/Analysis configuration is imported manually to the NovaSeq X Series instrument through the sample sheet. The mode uses EdgeOS (on the instrument or computer) to start the run. The downstream secondary analysis is done using the onboard DRAGEN module.
[2] The Cloud run mode sends the Run/Analysis configuration to ICA. This configuration is downloaded to the NovaSeq X Series instrument through ICA to start the run. The downstream secondary analysis is done in the cloud.
[3] The custom value must correspond to the longest index sequence of the samples in the pools in the library tube strip. Otherwise, the planned run creation fails and an error message displays.

6. Select Validate Run Setup and Create Planned Run to trigger the automation script. The script performs the following actions:
   - Validates the parameters entered on the Record Details screen.
   - Creates the planned run and sends it to ICA when the Run Mode is Cloud.
   - Generates the sample sheet and attaches it to the placeholder in the Files area on the Record Details screen for all Run Mode types.
7. Select Next Steps.
   On the Assign Next Steps screen, the next step for the pooled samples is set to the AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0) step.
8. Select Finish Step to advance the pooled samples to the AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0) step. For more information on how to start the sequencing run for different run modes, refer to *NovaSeq X Series Integration v1.0 Configuration and User Interaction* on page 99.

Step 5: AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0)

This protocol contains the AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0) step. This step is fully automated.

The integration starts the step automatically and data from the run is parsed back into Clarity LIMS. User interaction is not required, but you can review the stages of the step in Clarity LIMS.

Review Run Data

Read summary metrics are recorded for the library pool. After the run is complete, open the step and review these metrics in the Step Details section and the Sample Details table.

Step Details Section

The following values populate the master step fields:

- Run Name
- Run Status
- Output Folder
- Current Read
- Current Cycle
- Library Tube Barcode
- Flow Cell ID
- Flow Cell Side
- Flow Cell Type
- Flow Cell Part Number
- Flow Cell Lot Number
- Flow Cell Expiration Date
- Instrument ID
- Instrument Type
- Instrument Control Software Version
- Sequencing Log

Sample Details Table

Summary metrics populate the following global custom fields:

- % Bases >=Q30 R1
- % Bases >=Q30 R2
- % Error Rate R1
- % Error Rate R2

- Yield (Gb) R1
- Yield (Gb) R2
- Reads PF R1
- Reads PF R2
- %PF R1
- %PF R2
- % Aligned R1
- % Aligned R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- Cluster Density R1
- Cluster Density R2

Troubleshooting

If an automation trigger does not appear to run its corresponding scripts, refer to the *Troubleshooting Automation* section in the Clarity LIMS API & Database documentation.

Automated Step Does Not Start

If the AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0) step does not start, Clarity LIMS is likely not receiving sequencing run events from BaseSpace Sequence Hub correctly. Check for the following issues:

1. Make sure that Illumina Run Manager is configured properly as follows.
   a. Before you start the run, in Illumina Run Manager, select the appropriate BaseSpace Sequence Hub region in the Hosting location drop-down list.
   b. Under Run Settings, check the boxes next to Cloud run monitoring and Cloud run storage.
2. Make sure that the BaseSpace Sequence Hub code feature StratusRegionalEventsDisabled is turned off for the BaseSpace Sequence Hub region. If the feature is on, contact Illumina Support to turn it off. Make sure that the correct BaseSpace Sequence Hub region is selected.

Automated Step Starts, But Does Not Complete

If the AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0) step starts, but does not complete, do as follows.

1. Log in to the default user account and use one of the following methods to open the in progress step in Clarity LIMS:
   a. In Lab View, find the step in the Recent Activities pane.
   b. Search for the step in Clarity LIMS using the library tube strip barcode as the search term.
2. On the Record Details screen, the Sequencing Log multiline text field contains logging information. If unable to reach the Record Details screen, or if the Sequencing Log field does not contain enough information to resolve the issue, contact Illumina Support. Provide the relevant information from the troubleshooting steps already performed.

Validate Analysis Configuration Automation Check Fails

The Validate Analysis Configuration automation check is in the Make Bulk Pool (upon pooling) and Load to Library Tube Strip (upon step entry) steps. If a failure happens, an error message displays and the step can be aborted, or you might be prevented from continuing the step. This check can fail for the following reasons:

- The analysis configuration after library pooling or during the planned run creation exceeds the configuration limit. Resolve this error as follows.
  - If the error occurs on the Make Bulk Pool step, reduce the number of analysis configurations for a pool. To reduce the number, reorganize the samples for the pooling step (eg, by pooling samples that have similar configurations together).
  - If the error occurs on the Load to Library Tube step, reduce the number of analysis configurations for the planned run by reorganizing the samples for multiple runs instead of a single run.
- ACTs of samples in the same pool or planned run must have the same run mode (Local or Cloud). Resolve this error as follows.
  1. Abort the step and remove samples with ACTs that have conflicting run modes from the Ice Bucket.
  2. Make sure that all remaining samples in the Ice Bucket have ACTs with the same run modes.
  3. Select Begin Work to continue the step.
- ACTs of samples in the same pool or planned run that have the same secondary analysis must have the same analysis version. Resolve this error as follows.
  - If the error occurs on the Make Bulk Pool step, pool the samples again. Make sure that samples with the same secondary analysis, but conflicting analysis versions, are in different pools.

  - If the error occurs on the Load to Library Tube step, reorganize the samples for the planned run. Make sure that samples with the same secondary analysis, but conflicting analysis versions, are in different runs.

- ACTs of samples in the same pool or planned run that have the same secondary analysis must have the same analysis settings. The analysis setting fields can differ for a different secondary analysis and are configured in BaseSpace Sequence Hub when the ACT is created. Resolve this error as follows.
  1. If the error occurs on the Make Bulk Pool step, pool the samples again. Make sure that samples with the same secondary analysis, but conflicting analysis settings, are in different pools.
  2. If the error occurs on the Load to Library Tube step, reorganize the samples for the planned run. Make sure that samples with the same secondary analysis, but conflicting analysis settings, are in different runs.

NovaSeq X Series Integration v1.0 Release Notes

These Release Notes describe the key changes to software components for the Clarity LIMS NovaSeq X Series Integration Package version 1.0.

Compatibility

This version of the integration package is compatible with the following software:

- BaseSpace Clarity LIMS v5.3 and later
- NGS Extensions Package v5.22 and later
- Illumina Preset Protocols (IPP) v2.5.0 and later
- NovaSeq X Series Operating Software v1.0.0

New Features

Features provided with this release of the integration package are as follows.

- Provides integration between the NovaSeq X Series instrument and Clarity LIMS.
- Supports Local and Cloud run modes with automated tracking of the sequencing run and parsing of run statistics to Clarity LIMS.
- Supports the configuration of multiple secondary analyses on a sequencing run with the Analysis Configuration Template (ACT). This template can be configured using BaseSpace Sequence Hub.
- Generates the v2 sample sheet.
- Delivers the NovaSeq X Series Sequencing v1.0 workflow directly from IPP v2.5.

Defects Fixed

None

Known Limitations

- This integration does not track the analysis step. You must log in to BaseSpace Sequence Hub to view the cloud analysis status and results. For local analysis, the secondary analysis results are located in the external storage configured in Illumina Run Manager.
- The Local run mode does not support custom library prep and index adapter kits.

Known Issues

- On the Make Bulk Pool step, the log displays a warning when the Calculate Volume automation is triggered and at least one pool consists of multiple inputs. This issue is caused by the output custom field being reset multiple times at the end of the automation. This issue does not affect the volume calculation.

NovaSeq 6000Dx Integration

The Illumina NovaSeq 6000Dx integration provides preconfigured workflows that map to established lab protocols and steps used with NovaSeq 6000Dx sequencing instruments. The integration also supports the associated library prep kits, reagent kits, and assays used in these protocols.

NovaSeq 6000Dx Integration v1.2 Configuration and User Interaction

Illumina NovaSeqDx Integration Package v1.2 supports the integration of Clarity LIMS to Illumina NovaSeq 6000Dx instruments.

For instructions on validating and troubleshooting Illumina NovaSeqDx Integration Package v1.2, refer to *NovaSeq 6000Dx Integration v1.2 Validation and Troubleshooting* on page 59.

> The configuration provided in this integration has been established to support NovaSeq 6000Dx lab processes. Any configuration changes to protocols or workflows—including renaming protocols, steps, and fields—could break the process.

Overview

The integration includes the following features:

- Preconfigured NovaSeqDx v1.2 workflow that maps to lab protocols and instrument runs.
- The following preconfigured protocols:
  - Run Format (NovaSeqDx v1.2)
  - NovaSeqDx Standard (NovaSeqDx v1.2) (supports the loading of pooled libraries into a library tube)
  - NovaSeqDx Xp (NovaSeqDx v1.2) (supports individual lane loading on the NovaSeq)
  - AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2)
- Automated generation of v2 sample sheet. This file is automatically uploaded to the instrument via the API.
- Automatic validation of run setup information. This information is used to create the run recipe and initiate the run.
- Automated tracking of the NovaSeqDx sequencing run and parsing of run statistics into Clarity LIMS, including the following features:
  - Progress and metrics of sequencing run
  - Per-instrument sequencing runs (tracked as part of the Run ID field value)

- Sequencing run parameters
- Sequencing run data directory location
- Real-Time Analysis v3 (RTA3) version and other run-specific information
- [Optional] Preconfigured Library Prep Validation v2.3.1 workflow used for validation purposes only. The workflow contains a single-step protocol that models the library prep required to produce normalized libraries that are ready for the NovaSeqDx v1.2 workflow. For more information, refer to *NovaSeq 6000Dx Integration v1.2 Validation and Troubleshooting* on page 59. The Library Prep Validation v2.3.1 workflow is available in the Illumina Preset Protocols (IPP) v2.6 package.

Prerequisites and Assumptions

Samples enter the NovaSeqDx v1.2 workflow as normalized libraries. It is assumed that the following steps have completed before samples are assigned to the workflow:

- Samples have been accessioned into the Clarity LIMS.
- Samples have been run through QC and library prep.
- Samples have been normalized, and the value is captured in a field called Normalized Molarity (nM).

For more information on sample accessioning, refer to the following sections of the Getting Started section of the Clarity LIMS Core Functionality documentation:

- *Sample Accessioning*
- *Using Sample Lists to Upload and Modify Samples*

You can assign samples to workflows automatically, using a routing script, or manually—from the Projects & Samples dashboard. Refer to *Assign and Process Samples* in the Clarity LIMS Core Functionality documentation.

Workflows, Protocols, and Steps

The Illumina NovaSeq 6000Dx Integration Package v1.2 includes the following workflows:

- NovaSeqDx v1.2
- Library Prep Validation v2.3.1 (optional, but recommended for validation purposes)

The following table describes the protocols and steps included in these workflows.

Table 38 NovaSeq 6000Dx Integration Package v1.2 Protocols and Steps

Protocol Name	Protocol Purpose	Protocol Steps
Library Prep Validation v2.3.1 Workflow		

Protocol Name	Protocol Purpose	Protocol Steps
Library Prep Validation v2.3.1	Included for validation purposes only, this protocol models the library prep steps required to advance samples to the Run Format (NovaSeqDx v1.2) protocol. The protocol contains a single step - Library Prep Validation v2.3.1. After this step, a routing script sends the samples to the first step of the NovaSeqDx v1.2 workflow - Define Run Format (NovaSeqDx v1.2).	1. Library Prep Validation v2.3.1
	NovaSeqDx v1.2 Workflow	
Run Format (NovaSeqDx v1.2)	Allows for the assignment of per sample values for the following properties: • NovaSeqDx Run Mode: Select either RUO or DX. • Loading Workflow Type: Select either NovaSeq Standard or NovaSeq Xp. • Normalized Molarity (nM): Enter a value for each sample. • Flowcell Type: Select from options SP, S1, S2, or S4. • Final Loading Concentration (pM): Select from options 225 (PCR-free workflows) or 400 (Nano workflows), or enter a different value. Compares the Normalized Molarity value of each sample with the Minimum Molarity value. Validates user selection on NovaSeqDx Run Mode, Loading Workflow Type and Flowcell Type. Routing script sends samples to the NovaSeqDx Standard or NovaSeqDx Xp protocol, according to the selected Loading Workflow Type. Samples with Normalized Molarity less than Minimum Molarity are removed from the workflow.	1. Define Run Format (NovaSeqDx v1.2)

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Protocol Name	Protocol Purpose	Protocol Steps
NovaSeqDx Standard (NovaSeqDx v1.2)	Samples are pooled and added to the library tube in preparation for the NovaSeq 6000Dx run. The run setup information is validated and a sample sheet is generated. Routing script sends the library tube to the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2) protocol.	1. Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.2) 2. Dilute and Denature (NovaSeqDx v1.2)
NovaSeqDx Xp (NovaSeqDx v1.2)	Samples are pooled and added to lanes on the NovaSeq 6000Dx flow cell. The option selected in the Define Run Format (NovaSeqDx v1.2) step determines the flow cell type. The run setup information is validated and a sample sheet is generated. Routing script sends flow cell to the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2) protocol.	1. Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.2) 2. Dilute, Denature & ExAmp (NovaSeqDx v1.2) 3. Load to Flowcell (NovaSeqDx v1.2)
AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2)	All samples complete the workflow by going through this protocol. This protocol contains one fully automated step. Do not add samples to the Ice Bucket or start the step. The integration starts the step automatically.	1. AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2)

Validation Workflow

The Library Prep Validation v2.3.1 workflow allows for validation of the system after installation is complete. For details, refer to *NovaSeq 6000Dx Integration v1.2 Validation and Troubleshooting* on page 59.

Step Automations

The following table lists the step automations included in the NovaSeqDx v1.2 workflow. Automation details are provided in the documentation for each step.

For details on the validation workflow, refer to *NovaSeq 6000Dx Integration v1.2 Validation and Troubleshooting* on page 59.

Table 39 NovaSeq 6000Dx v1.2 Workflow Step Automations

Protocol	Step	Automation
1. Run Format (NovaSeqDx v1.2)	1. Define Run Format (NovaSeqDx v1.2)	Routing Script Set Next Steps
2. NovaSeqDx Standard (NovaSeqDx v1.2)	1. Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.2)	Calculate Volumes Set Next Step Validate Inputs Flowcell Type and Single Pool Validate Single NovaSeqDx Run Mode Validate Unique Indexes (Not used. The core Clarity LIMS configuration for pooling handles this functionality.)
	2. Dilute and Denature (NovaSeqDx v1.2)	Routing Script Validate Library Tube Barcode Validate Run Setup and Generate Sample Sheet Validate Single Input
3. NovaSeqDx Xp (NovaSeqDx v1.2)	1. Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.2)	Calculate Volumes Set Next Step Validate Inputs Flowcell Type and Single Pool Validate Unique Indexes (Not used. The core Clarity LIMS configuration for pooling handles this functionality.)
	2. Dilute, Denature & ExAmp (NovaSeqDx v1.2)	Calculate Volumes Set Next Step Validate Inputs Flowcell Type
	3. Load to Flowcell (NovaSeqDx v1.2)	Validate Flowcell Barcode Validate Inputs and Selected Container Validate Run Setup and Generate Sample Sheet

Protocol	Step	Automation
4. AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2)	1. AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2)	Update Lane Number Set Next Steps (Not used. By default, the Sequencer API determines this functionality. If necessary, you can enable the automation and use it to override the next step behavior used by the Sequencer API.)

Protocol 1: Run Format (NovaSeqDx v1.2)

This protocol sets the Loading Workflow Type and allows the choice of the appropriate Flowcell Type and Final Loading Concentration (pM). After the protocol, a routing script sends the normalized libraries to either the NovaSeqDx Standard (NovaSeqDx v1.2) or the NovaSeqDx Xp (NovaSeqDx v1.2) protocol.

This protocol contains one step: Define Run Format (NovaSeqDx v1.2).

Step 1. Define Run Format (NovaSeqDx v1.2)

Step input: Normalized Template Plate (NTP, normalized libraries)

Step output: None

Set Next Steps Automation

Automatically triggered on exit of the Record Details screen, this automation completes the following actions:

- Sets the next step for samples to REMOVE:

```
nextStep = ::REMOVE::
```

- Checks NovaSeqDx Run Mode, Loading Workflow Type, and Flowcell Type. Only the NovaSeq Standard with the S2/S4 flow cell is supported for the NovaSeqDx Run Mode:

```
if (input.::NovaSeqDx Run Mode:: == ::DX::) {
    if (input.::Loading Workflow Type:: == ::NovaSeq Xp::) {
        fail(::Invalid option selected. NovaSeq Standard shall be selected for DX
        mode.::);
    };
    if (input.::Flowcell Type:: == ::SP:: || input.::Flowcell Type:: == ::S1::) {
        fail(::Invalid option selected. Only S2 or S4 flowcell shall be selected
        for DX mode.::);
    };
};
```


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- Calculates the Minimum Molarity with the following formula:

```
input.::Minimum Molarity (nM):: = (5 * input.::Final Loading Concentration
(pM)::)/1000
```

- Checks Normalized Molarity value. For samples with no Normalized Molarity value (eg, empty value, not including 0), generates an error message informing that the field cannot be empty:

```
if (!input.hasValue(::Normalized Molarity (nM)::)) {
  fail(::The Normalized Molarity cannot be empty.::) ;
}
```

- Compares the Normalized Molarity value of each sample with the Minimum Molarity value. If the Normalized Molarity value is lower than the Minimum Molarity value, this sets the Loading Workflow Type of the sample to [Remove from workflow]. The automation also records a message in the Warning field for the sample:

```
else if (input.::Normalized Molarity (nM):: < input.::Minimum Molarity (nM)::) {
    input.::Warning:: = ::The Normalized Molarity is too low.::;
    input.::Loading Workflow Type:: = ::[Remove from workflow]::;
} else {
    input.::Warning:: = ::n/a::
}
```

At this point, the following options are available:

- Correct the NovaSeqDx Run Mode and Normalized Molarity value on the Record Details screen. Edit the Loading Workflow Type field and set it to NovaSeq Standard or NovaSeq Xp, as applicable.
- Complete the protocol without correcting the Normalized Molarity value. In this case, those samples are removed from the workflow.

Routing Script Automation

Automatically triggered on exit of the step, this automation invokes the changeWorkflow script, which routes step inputs appropriately.

- Samples with Loading Workflow Type field value = NovaSeq Standard are routed to the NovaSeqDx v1.2 workflow. Then, the samples are queued for the Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.2) step.
- Samples with Loading Workflow Type field value = NovaSeq Xp are routed to the NovaSeqDx v1.2 workflow. Then, the samples are queued for the Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.2) step.

Default automation command line is as follows.

```
bash -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar
```

```
-u {username} -p {password} -i {stepURI:v2} -l {compoundOutputFileLuid0}
script:changeWorkflow \
\
--FIELD_NAME 'Loading Workflow Type' \
--FIELD_VALUE 'NovaSeq Standard' \
--WORKFLOW 'NovaSeqDx v1.2' \
--STEP 'Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.2)' \
--INPUTS_OR_OUTPUTS 'INPUTS' \
\
--FIELD_NAME 'Loading Workflow Type' \
--FIELD_VALUE 'NovaSeq Xp' \
--WORKFLOW 'NovaSeqDx v1.2' \
--STEP 'Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.2)' \
--INPUTS_OR_OUTPUTS 'INPUTS'"
```

Master Step Fields

There are four fields defined on the Define Run Format (NovaSeqDx v1.2) master step.

The following table lists field configuration details.

Table 40 Define Run Format (NovaSeqDx v1.2) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Comment	Multiline Text		None
Flowcell Type	Text Dropdown	Required Field Custom Entries	Presets • SP • S1 • S2 • S4
Instruction	Text	Read Only	Default • Add Flowcell Type, Loading Workflow Type, and NovaSeqDx Run Mode
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp

Global Fields

The following table lists the global custom fields that are configured to display on the Define Run Format (NovaSeqDx v1.2) step.

Table 41 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
Adjusted Per Sample Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Final Loading Concentration (pM)	Numeric Dropdown	Required Field Custom Entries	Presets • 225 • 400 Decimal places displayed = 0
Flowcell Type	Text Dropdown	Required Field	Presets • SP • S1 • S2 • S4
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
Minimum Molarity (nM)	Numeric		Decimal places displayed = 2
Normalized Molarity (nM)	Numeric		Decimal places displayed = 2
NovaSeqDx Run Mode	Text Dropdown	Required Field	Presets • DX • RUO
Per Sample Volume (µl)	Numeric	Read Only	Decimal places displayed = 2

Field Name	Field Type	Options	Additional Options and Dropdown Items
Warning	Text Dropdown	Read Only Custom Entries	Presets • The Normalized Molarity (nM) is too low. • n/a

User Interaction

Refer to the Step 1: Define Run Format (NovaSeqDx v1.2) section in *NovaSeq 6000Dx Integration v1.2 Validation and Troubleshooting* on page 59.

Protocol 2: NovaSeqDx Standard (NovaSeqDx v1.2)

Samples are routed to this protocol when their Loading Workflow Type value is set to NovaSeq Standard. Samples are pooled and added to a library tube in preparation for the NovaSeqDx Run.

At the end of this protocol, a routing script sends the library tube to the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2) protocol.

This protocol contains the following two steps:

- Step 1: Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.2)
- Step 2: Dilute and Denature (NovaSeqDx v1.2)

Step 1: Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.2)

In this step, libraries are placed manually into a single pool. Resuspension buffer and reagents are added.

Create only one pool per step.

Step input: NTP (normalized libraries)

Step output: Bulk pool

Validate Unique Indexes automation is not used. The core Clarity LIMS configuration for pooling handles this functionality.

Validate Single NovaSeqDx Run Mode Automation

The Validate Single NovaSeqDx Run Mode automation is triggered on entry of the step. The following automation checks that all input samples have the same NovaSeqDx Run Mode assigned:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar \
```

```
script:validate_same_analyte_udf_value_for_all_inputs \
-i {stepURI:v2} \
-u {username} \
-p {password} \
-l {compoundOutputFileLuid1} \
-f 'NovaSeqDx Run Mode'"
```

Validate Inputs Flowcell Type and Single Pool Automation

Automatically triggered on exit of the Pooling screen, this automation completes the following actions:

- Checks that all samples in the pool have the same Flowcell Type assigned to them.
- Checks that only one pool has been created.
- Changes the value of error and logging messages to reference the type of pool passed as bulk pool.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar \
script:validate_flowcell_for_input_pools \
-i {stepURI:v2} \
-u {username} \
-p {password} \
-l {compoundOutputFileLuid1} \
-validateSingleOutput true \
-poolType bulk"
```

Calculate Volumes Automation

Automatically triggered when you select the Calculate Volumes button on the Record Details screen, this automation completes the following actions:

- Calculates the number of samples in the pool.

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: + 1
```

- Sets the value of the Bulk Pool Volume (µl), and PhiX Volume (µl) field based on the selected Flowcell Type.

```
step.::PhiX Volume (ul):: = !step.hasValue(::% PhiX (2.5nM) Spike-In::) ? 0 :
step.::% PhiX (2.5nM) Spike-In::;
if (input.::Flowcell Type:: == ::SP::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 100;
step.::PhiX Volume (ul):: *= 0.6
};
if (input.::Flowcell Type:: == ::S1::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 100;
```

```
step.::PhiX Volume (ul):: *= 0.6
};
if (input.::Flowcell Type:: == ::S2::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 150;
step.::PhiX Volume (ul):: *= 0.9
};
if (input.::Flowcell Type:: == ::S4::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 310;
step.::PhiX Volume (ul):: *= 1.9
};
if (step.::PhiX Volume (ul):: == 0) {
step.::PhiX Volume (ul):: = ::::
};
```

- Calculates the Per Sample Volume (µl) to be added to the pool.

```
input.::Per Sample Volume (ul):: = ( ( (input.::Final Loading Concentration
(pM):: * 5 / 1000) / input.::Normalized Molarity (nM):: ) * step.::Bulk Pool
Volume (ul):: ) / step.::Number of Samples in Pool::
```

> For accurate pipetting of each sample in a pool for sequencing, the Per Sample Volume (µl) value must be equal to or higher than the Minimum Per Sample Volume (µl). The default value (set at 5) can be edited.

Assuming the default Minimum Per Sample Volume (µl) value of 5, this automation completes the following steps for a given batch:

1. If the smallest Per Sample Volume (µl) value is less than 5, automatically assigns a value of 5 to the Adjusted Per Sample Volume (µl) field of the sample.
2. Adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

- Calculates the Total Sample Volume (µl) field value.

```
step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: +
input.::Adjusted Per Sample Volume (ul)::
```

- If the Total Sample Volume is less than the Bulk Pool Volume, this automation calculates the RSB Volume (µl) field value.

```
if (step.::Total Sample Volume (ul):: >= step.::Bulk Pool Volume (ul)::) {
output.::RSB Volume (ul):: = 0
}
else {
output.::RSB Volume (ul):: = step.::Bulk Pool Volume (ul):: - step.::Total
Sample Volume (ul)::
} ;
```


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- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs.

```
output.::Flowcell Type:: = input.::Flowcell Type:: ;
output.::Loading Workflow Type:: = input.::Loading Workflow Type:: ;
```

- Sets the Volume of Pool to Denature (µl) value a calculates the NaOH Volume (µl) and Tris-HCl Volume (µl) values based on the Flowcell Type.

```
if ( input.::Flowcell Type:: == ::SP:: ) {
output.::Volume of Pool to Denature (ul):: = 100 ;
output.::NaOH Volume (ul):: = 25 ;
output.::Tris-HCl Volume (ul):: = 25
} ;
if ( input.::Flowcell Type:: == ::S1:: ) {
output.::Volume of Pool to Denature (ul):: = 100 ;
output.::NaOH Volume (ul):: = 25 ;
output.::Tris-HCl Volume (ul):: = 25
} ;
if ( input.::Flowcell Type:: == ::S2:: ) {
output.::Volume of Pool to Denature (ul):: = 150 ;
output.::NaOH Volume (ul):: = 37 ;
output.::Tris-HCl Volume (ul):: = 38
} ;
if ( input.::Flowcell Type:: == ::S4:: ) {
output.::Volume of Pool to Denature (ul):: = 310 ;
output.::NaOH Volume (ul):: = 77;
output.::Tris-HCl Volume (ul):: = 78
}
```

- Uses the `NovaSeq_Standard_Bulk_Pool1.csv`, `NovaSeq_Standard_Bulk_Pool2.csv`, and `NovaSeq_Standard_Bulk_Pool3.csv` template files to generate a single CSV file containing information about the pool and the samples it contains. The generated file is available for download in the Files section of the Record Details milestone in the Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.2) step.

```
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} \
   script:driver_file_generator \
      -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Standard_
      Bulk_Pool1.csv \
      -o 1.csv \
   script:driver_file_generator \
```

```
    -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Standard_
    Bulk_Pool2.csv \
    -o 2.csv \
  script:driver_file_generator \
    -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Standard_
    Bulk_Pool3.csv \
    -o 3.csv \
&& cat 1.csv 2.csv 3.csv > {compoundOutputFileLuid0}.csv
```

- Resets the Total Sample Volume (µl) and Number of Samples in Pool field values so that the automation is idempotent.

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: - 1;
step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: -
input.::Adjusted Per Sample Volume (ul)::
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, the following automation sets the next step for samples to ADVANCE, advancing them to the Dilute and Denature (NovaSeqDx v1.2) step in the protocol:

```
nextStep = ::ADVANCE::
```

The following automation copies the NovaSeqDx Run Mode values from the step inputs to the step outputs:

```
output.::NovaSeqDx Run Mode:: = input.::NovaSeqDx Run Mode::
```

Master Step Fields

The following fields are defined on the Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.2) step.

Table 42 Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.2) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
% PhiX (2.5 nM) Spike-In	Numeric		Range = 0–100
Bulk Pool Volume (µl) (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 2

Field Name	Field Type	Options	Additional Options and Dropdown Items
Number of Flowcells to Sequence	Numeric	Required Field	Range = 1–10 Decimal places displayed = 0
Minimum Per Sample Volume (µl)	Numeric	Required Field	Decimal places displayed = 2 Default • 5
Number of Samples in Pool (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 0 Default • 0
PhiX Volume (µl) (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 2
Total Sample Volume (µl) (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 2 Default • 0

Global Fields

The following table lists the global custom fields that are configured to display on the Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.2) step.

Table 43 Global Custom Fields Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
Flowcell Type	Text Dropdown	Required Field	Presets • SP • S1 • S2 • S4

Field Name	Field Type	Options	Additional Options and Dropdown Items
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
NaOH Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
RSB Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Tris-HCl Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Volume of Pool to Denature (µl) (Used in Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.2) step only. Displays on Record Details screen and in the generated CSV file.)	Numeric	Read Only	Decimal places displayed = 0

User Interaction

Refer to the Step 1: Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.2) section in *NovaSeq 6000Dx Integration v1.2 Validation and Troubleshooting* on page 59.

Step 2: Dilute and Denature (NovaSeqDx v1.2)

In this step, the addition of NaOH, Tris-HCl, and Resuspension Buffer (RSB) denatures and dilutes pooled samples. Manually place the pooled samples into the library tube for the NovaSeqDx Run.

In addition, this step validates the run setup information and generates the sample sheet file.

Step input: Bulk pool

Step output: Library tube

Validate Single Input Automation

Automatically triggered at the beginning of the step, this automation checks that there is only one container input to the step.

```
script:validateSampleCount -min 1 -max 1
```

Validate Library Tube Barcode Automation

Automatically triggered on exit of the Placement screen, this automation completes the following actions:

- Validates the library tube barcode to ensure it conforms to the following barcode masks:
  - DX mode: DX[0-9]{7}-[A-Z]{3}
  - RUO mode: [A-Z]{2}[0-9]{7}-[A-Z]{3}

```
if ((input.::NovaSeqDx Run Mode:: == ::DX:: && !output.container.name.matches
(::^DX[0-9]{7}-[A-Z]{3}$::)) || (input.::NovaSeqDx Run Mode:: == ::RUO:: &&
!output.container.name.matches(::[A-Z]{2}[0-9]{7}-[A-Z]{3}::))) {
fail(::Invalid Library Tube Barcode. Please verify and try again.::)
}
```

- Copies Flowcell Type and Loading Workflow Type field values from step inputs to outputs:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ;
output.::Loading Workflow Type:: = input.::Loading Workflow Type::
```

Validate Run Setup and Generate Sample Sheet Automation

Triggered by a button on the Record Details screen, this automation completes the following actions:

> The script definition portion of Validate Run Setup has been moved into the Validation Script custom field due to the 4000 character limit. If modification is required on Validate Run Setup script, perform the changes directly on the Validation Script custom field default value.

- Copies the Flowcell Type from the step input to the Run Mode field (hidden):

```
step.::Run Mode:: = input.::Flowcell Type::
```

- Validates the parameters entered on the Record Details screen, which is used to set up the run and generate the sample sheet file. The parameters are as follows. For more information, refer to the *Respond to Validation / Recipe Request Call from Instrument* section of *NovaSeq 6000Dx Run*.
  - Experiment Name only contains alphanumeric, dash, or underscore characters. Spaces are not permitted.
  - When Workflow Type = No Index, both Index Read 1 and Index Read 2 must be zero.
  - When Workflow Type = Single Index, Index Read 1 must be greater than zero, and Index Read 2 must be zero.
  - When Workflow Type = Dual Index, both Index Read 1 and Index Read 2 must be greater than zero.

```
if(!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) {
```

```
fail(::Experiment Name contains prohibited characters. Allowed characters are:
a-z, A-Z, 0-9, -, and _::)
};
if (step.::Workflow Type:: == ::No Index::) {
if (step.::Index Read 1:: != 0 || step.::Index Read 2:: != 0) {
fail(::Index Read 1 and Index Read 2 must be 0 if No Index is selected.::)
}
} else if (step.::Workflow Type:: == ::Single Index::) {
if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) {
fail(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if Single
Index is selected.::)
}
} else {
if (step.::Index Read 1:: == 0 || step.::Index Read 2:: == 0) {
fail(::Index Read 1 and Index Read 2 must be greater than 0 if Dual Index is
selected.::)
}
};
```

- Checks the Paired End and Read 2 Cycles field values as follows.
  - If Paired End = True, both Read 1 Cycles and Read 2 Cycles must be greater than 0.
  - If Paired End = False, Read 2 Cycles must be zero.

```
if (step.::Paired End::.toBoolean()) {
   if (step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) {
      fail(::Read 1 Cycles and Read 2 Cycles must be greater than 0 if Paired
      End is True.::)
   }
} else {
   if (step.::Read 2 Cycles:: != 0) {
      fail(::Read 2 Cycles must be 0 if Paired End is False.::)
   }
};
```

- Checks the Flowcell field value as follows.
  - If Flowcell Type value is not SP, checks that the values of Read 1 Cycles and Read 2 Cycles are each 151 or less. If the value is greater than 151, an error message is generated.

```
if (input.::Flowcell Type::!=::SP:: && step.::Read 1 Cycles:: > 151) {
fail(::Read 1 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
};
if (input.::Flowcell Type::!=::SP:: && step.::Read 2 Cycles:: > 151) {
fail(::Read 2 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
```


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```
};
```

- Validate Analysis Software Version to ensure it is a valid version string.

```
if (step.hasValue(::Analysis Software Version::) && !step.::Analysis Software
Version::.matches(::^(\\d+)(\\.\\d+)*$::)) {
fail(::Analysis Software Version contains prohibited characters. Allowed
characters are 0-9 and period. It shall start and end with numbers and separated
by single period e.g. 3.8.4::)
};
```

- Validate Override Cycles value to contain only Y, N, I, U, 0-9, and semicolon characters.

```
if (step.hasValue(::Override Cycles::) && !step.::Override Cycles::.matches(::
[YNIU0-9;]+::)) {
fail(::Override Cycles contains prohibited characters. Allowed characters are:
Y, N, I, U, 0-9 and ;. Example: N1Y150;I8;I7N1;Y141U10.::)
};
```

- Sets the next step for samples to REMOVE:

```
nextStep = ::REMOVE::
```

- Generates the V2 sample sheet and attaches it to the step. For more information, refer to *Sample Sheet Generation*.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeqDx_BCL2FASTQ_
Samplesheet_v2.csv \
-o {compoundOutputFileLuid0}.csv \
-q true \
-destLIMSID {compoundOutputFileLuid0} \
-l {compoundOutputFileLuid1}"
```

Routing Script Automation

Automatically triggered on exit of the step, this automation invokes the changeWorkflow script, which routes step outputs to the NovaSeqDx v1.2 workflow, and queues them for the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2) step.

The default automation command line is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar
-u {username} -p {password} -i {stepURI:v2} -l {compoundOutputFileLuid1}
script:changeWorkflow \
```


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```
--FIELD_NAME 'N/A' \
--FIELD_VALUE 'N/A' \
--WORKFLOW 'NovaSeqDx v1.2' \
--STEP 'AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2)' \
--INPUTS_OR_OUTPUTS 'OUTPUTS'"
```

Master Step Fields

The following table lists field configuration details for the fields that are defined on the Dilute and Denature (NovaSeqDx v1.2) step. These fields are required for sample sheet and JSON file generation.

Table 44 Dilute and Denature (NovaSeqDx v1.2) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Analysis Software Version	Text		
BaseSpace Sequence Hub Configuration	Text Dropdown	Required Field	Not Used Run Monitoring Only Run Monitoring and Storage
Custom Recipe Path	Text		
Experiment Name	Text	Required Field	
Index Read 1	Numeric Dropdown	Required Field Custom Entries	Range = 0–20 Decimal places displayed = 0 Presets • 0 • 6 • 8
Index Read 2	Numeric Dropdown	Required Field Custom Entries	Range = 0–20 Decimal places displayed = 0 Presets • 0 • 6 • 8
Output Folder	Text	Required Field	
Override Cycles	Text		

Field Name	Field Type	Options	Additional Options and Dropdown Items
Paired End	Text Dropdown	Required Field	Presets • True • False
Read 1 Cycle	Numeric Dropdown	Required Field Custom Entries	Range = 1–251 Decimal places displayed = 0 Presets • 251* • 151 • 101 • 51 *Value of 251 is only supported for SP flow cell type. For all other cell types, maximum value is 151.
Read 2 Cycle	Numeric Dropdown	Required Field Custom Entries	Range = 0–251 Decimal places displayed = 0 Presets • 251* • 151 • 101 • 51 *Value of 251 is only supported for SP flow cell type. For all other cell types, maximum value is 151.
Run Mode (Not displayed in user interface)	Text Dropdown	Read Only	Presets • SP • S1 • S2 • S4
Use Custom Index Read 1 Primer	Toggle Switch		Default • None Set

For Research Use Only. Not for use in diagnostic procedures.

Field Name	Field Type	Options	Additional Options and Dropdown Items
Use Custom Read 1 Primer	Toggle Switch		Default • None Set
Use Custom Read 2 Primer	Toggle Switch		Default • None Set
Use Custom Recipe	Toggle Switch	Required Field	Default • No
Validation Script	Multiline Text	Required Field Read Only	Default value is provided in the drop-down section that follows the table. Do not remove this field as it is used by Validate Run Setup and Generate Sample Sheet automation script.
Workflow	Text	Read Only	Default • GenerateFASTQ
Workflow Type	Text Dropdown	Required Field	Presets • No Index • Single Index • Dual Index • Custom

Validation Script

```
step.::Run Mode:: = input.::Flowcell Type::; if (!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) { fail(::Experiment Name contains prohibited characters. Allowed characters are: a-z, A-Z, 0-9, -, and _::) }; if (step.::Workflow Type:: == ::No Index::) { if (step.::Index Read 1:: != 0 || step.::Index Read 2:: != 0) { fail(::Index Read 1 and Index Read 2 must be 0 if No Index is selected.::) } } else if (step.::Workflow Type:: == ::Single Index::) { if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) { fail(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if Single Index is selected.::) } } else { if (step.::Index Read 1:: == 0 || step.::Index
```

```
Read 2:: == 0) { fail(::Index Read 1 and Index Read 2 must be greater than 0 if
Dual Index is selected.::) } }; if (step.::Paired End::.toBoolean()) { if
(step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) { fail(::Read 1
Cycles and Read 2 Cycles must be greater than 0 if Paired End is True.::) } }
else { if (step.::Read 2 Cycles:: != 0) { fail(::Read 2 Cycles must be 0 if
Paired End is False.::) } }; if (input.::Flowcell Type:: != ::SP:: &&
step.::Read 1 Cycles:: > 151) { fail(::Read 1 Cycles must not be larger than 151
if it is not SPrime Flowcell::) }; if (input.::Flowcell Type:: != ::SP:: &&
step.::Read 2 Cycles:: > 151) { fail(::Read 2 Cycles must not be larger than 151
if it is not SPrime Flowcell::) }; if (step.hasValue(::Analysis Software
Version::) && !step.::Analysis Software Version::.matches(::^(\\d+)
(\\.\\d+)*$::)) { fail(::Analysis Software Version contains prohibited
characters. Allowed characters are 0-9 and period. It shall start and end with
numbers and separated by single period e.g. 3.8.4::) }; if (step.hasValue
(::Override Cycles::) && !step.::Override Cycles::.matches(::[YNIU0-9;]+::)) {
fail(::Override Cycles contains prohibited characters. Allowed characters are:
Y, N, I, U, 0-9 and ;. Example: N1Y150;I8;I7N1;Y141U10.::) }; nextStep =
::REMOVE::
```

Global Fields

The following table lists the global custom fields that are configured to display on the Dilute and Denature (NovaSeqDx v1.2) step.

Table 45 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
Flowcell Type	Text Dropdown	Required Field	Presets • SP • S1 • S2 • S4
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User Interaction

Refer to the Step 2: Dilute and Denature (NovaSeqDx v1.2) section in *NovaSeq 6000Dx Integration v1.2 Validation and Troubleshooting* on page 59.

Protocol 3: NovaSeqDx Xp (NovaSeqDx v1.2)

Samples are routed to this protocol when their Loading Workflow Type value is set to NovaSeq Xp.

Samples are pooled and added to lanes on the NovaSeqDx flow cell type selected in the Define Run Format (NovaSeqDx v1.2) step. At the end of this protocol, the flow cell is sent to the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2) protocol.

This protocol contains the following three steps:

- Step 1: Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.2)
- Step 2: Dilute, Denature & ExAmp (NovaSeqDx v1.2)
- Step 3: Load to Flowcell (NovaSeqDx v1.2)

Step 1: Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.2)

Manually place libraries into a pool.

Create only one pool per step.

Step input: NTP (normalized libraries)

Step output: Bulk pool

Validate Unique Indexes automation is not used. The core Clarity LIMS configuration for pooling handles this functionality.

Validate Inputs Flowcell Type and Single Pool Automation

Automatically triggered on exit of the Pooling screen, this automation completes the following actions:

- Checks that all samples in the pool have the same Flowcell Type assigned to them.
- Checks that only one pool has been created.
- Changes the value of error and logging messages to reference the type of pool passed as 'bulk' pool.

```
/opt/gls/clarity/bin/java \
-jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar \
script:validate_flowcell_for_input_pools \
-i {stepURI:v2} \
-u {username} \
-p {password} \
```

```
-l {compoundOutputFileLuid1} \
-validateSingleOutput true \
-poolType bulk"
```

Calculate Volumes Automation

Triggered when the Calculate Volumes button is selected on the Record Details screen, this automation completes the following actions:

- Calculates the number of samples in the pool:

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: + 1
```

- Sets the value of the Bulk Pool Volume (µl) and PhiX Volume (µl) field, based on the selected Flowcell Type:

```
step.::PhiX Volume (ul):: = !step.hasValue(::% PhiX (0.25nM) Spike-In::) ? 0 :
step.::% PhiX (0.25nM) Spike-In::;
if (input.::Flowcell Type:: == ::SP::) {
step.::Bulk Pool Volume (ul):: = step.'Number of Lanes to Sequence' * 18;
step.::PhiX Volume (ul):: *= 0.7;
};
if (input.::Flowcell Type:: == ::S1::) {
step.::Bulk Pool Volume (ul):: = step.'Number of Lanes to Sequence' * 18;
step.::PhiX Volume (ul):: *= 0.7;
};
if (input.::Flowcell Type:: == ::S2::) {
step.::Bulk Pool Volume (ul):: = step.'Number of Lanes to Sequence' * 22;
step.::PhiX Volume (ul):: *= 0.8;
};
if (input.::Flowcell Type:: == ::S4::) {
step.::Bulk Pool Volume (ul):: = step.'Number of Lanes to Sequence' * 30;
step.::PhiX Volume (ul):: *= 1.1;
};
if (step.::PhiX Volume (ul):: == 0) {
step.::PhiX Volume (ul):: = ::::;
};
```

- Calculates the Per Sample Volume (µl) to be added to the pool:

```
input.::Per Sample Volume (µl)::= (((input.::Final Loading Concentration (pM)::*
5/ 1000) / input.::Normalized Molarity (nM)::) * step.::Bulk Pool Volume (µl)::)
/ step.::Number of Samples in Pool::
```

To ensure accurate pipetting of each sample in a pool for sequencing, the Per Sample Volume (µl) value must be equal to or higher than the Minimum Per Sample Volume (µl). The default value (set at 5) can be edited.

Assuming the default Minimum Per Sample Volume (µl) value of 5, completes the following steps for a given batch:

1. If the smallest Per Sample Volume (µl) value is less than 5, automatically assigns a value of 5 to the sample Adjusted Per Sample Volume (µl) field.
2. Adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

- Calculates the Total Sample Volume (µl) field value:

```
step.::Total Sample Volume (µl):: = step.::Total Sample Volume (µl):: +
input.::Adjusted Per Sample Volume (µl)::
```

- If the Total Sample Volume is less than the Bulk Pool Volume, this automation calculates the RSB Volume (µl) field value:

```
if (step.::Total Sample Volume (ul):: >= step.::Bulk Pool Volume (ul)::) {
output.::RSB Volume (ul):: = 0
}
else {
    output.::RSB Volume (ul):: = step.::Bulk Pool Volume (ul):: - step.::Total
    Sample Volume (ul)::
} ;
```

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs:

```
output.::Flowcell Type::= input.::Flowcell Type::;output.::Loading Workflow
Type::= input.::Loading Workflow Type::;
```

- Uses the `NovaSeq_Xp_Bulk_Pool.csv` and `NovaSeq_Xp_Bulk_Pool2.csv` template files to generate a single CSV file containing information about the bulk pool and the samples it contains. The generated file is available for download in the Files segment of the Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.2) step.

```
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} \
    script:driver_file_generator \
        -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_Bulk_
        Pool.csv \
        -o {compoundOutputFileLuid0}.csv \
    script:driver_file_generator \
```

```
        -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_Bulk_
        Pool2.csv \
        -o append.csv \
&& cat append.csv >> {compoundOutputFileLuid0}.csv"
```

- Resets the Total Sample Volume (µl) and Number of Samples in Pool field values so that the automation is idempotent.

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: - 1;
step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: -
input.::Adjusted Per Sample Volume (ul)::
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation completes the following actions:

- Copies the Flowcell Type values from the step inputs to the step outputs.
- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ; nextStep = ::ADVANCE::
```

Master Step Fields

The fields in the following table are defined on the Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.2) step.

Table 46 Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.2) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
% PhiX (0.25nM) Spike-in	Numeric		Range = 0– 100
Bulk Pool Volume (µl) (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 2
Minimum Per Sample Volume (µl)	Numeric	Required Field	Default • 5 Decimal places displayed = 2
Number of Lanes to Sequence	Numeric	Required Field	Decimal places displayed = 0

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Field Name	Field Type	Options	Additional Options and Dropdown Items
Number of Samples in Pool (for calculation purposes, not displayed)	Numeric		Default • 0 Decimal places displayed = 0
PhiX Volume (µl) (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 2
Total Sample Volume (µl) (for calculation purposes, not displayed)	Numeric		Default • 0 Decimal places displayed = 0

Global Fields

The following table lists the global custom fields that are configured to display on the Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.2) step.

Table 47 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
RSB Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Flowcell Type	Text Dropdown	Required Field	Presets • SP • S1 • S2 • S4
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User Interaction

Refer to the Step 1: Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.2) section in *NovaSeq 6000Dx Integration v1.2 Validation and Troubleshooting*.

Step 2: Dilute, Denature & ExAmp (NovaSeqDx v1.2)

In this step, the addition of DPX, NaOH, Tris-HCl, and RSB denatures and dilutes pooled samples. Manually create working pools based on the number of lanes that you want to sequence.

Step input: Bulk pool

Step output: Working pool - variable number (choose how many working pools to create per bulk pool)

Validate Inputs Flowcell Type Automation

Automatically triggered at the beginning of the step, this automation invokes the `validate_flowcell_for_input_pools` script. This script completes the following actions:

- Checks the inputs to the step and validates that the Flowcell Type field is set to a valid value (SP, S1, S2, or S4). This automation also checks that all inputs have the same value for this field.
- The container type selected matches the value in the Flowcell Type field.

```
bash -l -c "/opt/gls/clarity/bin/java \
-jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar \
script:validate_flowcell_for_input_pools \
-i {stepURI:v2} \
-u {username} \
-p {password} \
-l {compoundOutputFileLuid1}"
```

Calculate Volumes Automation

Automatically triggered on entry to of the Record Details screen, this automation sets the value of the following fields based on the Flowcell Type:

- BP Aliquot Volume (µl)
- NaOH Volume (µl)
- Tris-HCl Volume (µl)
- DPX1 Volume (µl)
- DPX2 Volume (µl)
- DPX3 Volume (µl)
- Mastermix per Lane (µl)

```
if ( input.::Flowcell Type:: == ::SP:: ) {
output.::BP Aliquot Volume (µl):: = 18 ;
```

```
output.::NaOH Volume (µl):: = 4 ;
output.::Tris-HCl Volume (µl):: = 5 ;
step.::DPX1 Volume (µl):: = 126 ;
step.::DPX2 Volume (µl):: = 18 ;
step.::DPX3 Volume (µl):: = 66 ;
output.::Mastermix per Lane (µl):: = 63
};

if (input.::Flowcell Type:: == ::S1::) {
output.::BP Aliquot Volume (µl):: = 18;
output.::NaOH Volume (µl):: = 4 ;
output.::Tris-HCl Volume (µl):: = 5 ;
step.::DPX1 Volume (µl):: = 126;
step.::DPX2 Volume (µl):: = 18;
step.::DPX3 Volume (µl):: = 66;
output.::Mastermix per Lane (µl):: = 63
};
if (input.::Flowcell Type:: == ::S2::) {
output.::BP Aliquot Volume (µl):: = 22;
output.::NaOH Volume (µl):: = 5 ;
output.::Tris-HCl Volume (µl):: = 6 ;
step.::DPX1 Volume (µl):: = 126;
step.::DPX2 Volume (µl):: = 18;
step.::DPX3 Volume (µl):: = 66;
output.::Mastermix per Lane (µl):: = 77
};
if (input.::Flowcell Type:: == ::S4::) {
output.::BP Aliquot Volume (µl):: = 30;
output.::NaOH Volume (µl):: = 7 ;
output.::Tris-HCl Volume (µl):: = 8 ;
step.::DPX1 Volume (µl):: = 315;
step.::DPX2 Volume (µl):: = 45;
step.::DPX3 Volume (µl):: = 165;
output.::Mastermix per Lane (µl):: = 105
};
```

The automation also completes the following actions:

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs:

```
output.::Flowcell Type::= input.::Flowcell Type::;
```

```
output.::Loading Workflow Type::= input.::Loading Workflow Type::;
```

- Uses the `NovaSeq_Xp_Working_Pool.csv` and `NovaSeq_Xp_Working_Pool2.csv` template files to generate a single CSV file. This file contains information about the DPX volume and the volume of BP Aliquot, Mastermix, NaOH, and Tris-HCl to add per working pool. The generated file is available for download in the Files segment of the Dilute, Denature & ExAmp (NovaSeqDx v1.2) step.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} \
   script:driver_file_generator \
      -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_
      Working_Pool.csv \
      -o {compoundOutputFileLuid0}.csv \
   script:driver_file_generator \
      -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_
      Working_Pool2.csv \
      -o append.csv \
&& cat append.csv >> {compoundOutputFileLuid0}.csv"
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation:

- Copies the Flowcell Type values from the step inputs to the step outputs.
- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ; nextStep = ::ADVANCE::
```

Master Step Fields

The following table lists field configuration details for the fields that are defined on the Dilute, Denature & ExAmp (NovaSeqDx v1.2) step. A script sets these field values. They are not editable while running the step.

Table 48 Dilute, Denature & ExAmp (NovaSeqDx v1.2) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
DPX1 Volume (µl)	Numeric	Read Only	Decimal places displayed = 0
DPX2 Volume (µl)	Numeric	Read Only	Decimal places displayed = 0

Field Name	Field Type	Options	Additional Options and Dropdown Items
DPX3 Volume (µl)	Numeric	Read Only	Decimal places displayed = 0

Global Fields

The following table lists the global custom fields that are configured to display on the Dilute, Denature & ExAmp (NovaSeqDx v1.2) step.

Table 49 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
BP Aliquot Volume (µl)	Numeric	Read Only	• Decimal places displayed = 0
Flowcell Type	Text Dropdown	Required Field	Presets • SP • S1 • S2 • S4
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
Mastermix per Lane (µl)	Numeric	Read Only	Decimal places displayed = 0
NaOH Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Tris-HCl Volume (µl)	Numeric	Read Only	Decimal places displayed = 2

User Interaction

Refer to the Step 2: Dilute, Denature & ExAmp (NovaSeqDx v1.2) section in *NovaSeq 6000Dx Integration v1.2 Validation and Troubleshooting* on page 59.

Step 3: Load to Flowcell (NovaSeqDx v1.2)

In this step, scan the flow cell barcode into the Clarity LIMS and manually place the working pools into the lanes of the flow cell for the NovaSeqDx run. This step validates the run setup information and generates the sample sheet file.

Step input: Working pool

Step output: Flow cell (output containers: SP, S1, and S2 with 2 lanes, and S4 with 4 lanes)

Validate Inputs and Selected Container Automation

Automatically triggered at the beginning of the step, this automation invokes the validate_flowcell_for_input_pools and validateSelectedContainer scripts. These scripts validate the step inputs and the container selected by the user, as follows.

- Checks that the Flowcell Type field has been set to a valid value (SP, S1, S2, or S4) and that all inputs have the same value.
- Checks that the number of outputs matches the number of lanes on the selected flow cell type. If validation fails, an error message informs that the number of working pools does not match the number of lanes available on the flow cell.

The following table defines the number of samples allowed:

Flow Cell Type	Number of Working Pools
SP	2
S1	2
S2	2
S4	4

- Checks that the container type selected on entry to the Placement screen matches the value in the Flowcell Type field. If validation fails, an error message displays.

```
bash -l -c "/opt/gls/clarity/bin/java \
-jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar
\script:validate_flowcell_for_input_pools \
  -i {stepURI:v2} \
  -u {username} \
  -p {password} \
  -l {compoundOutputFileLuid1} \
  -validateSelectedContainer true"
```


For Research Use Only. Not for use in diagnostic procedures.

Validate Flowcell Barcode Automation

Automatically triggered on exit of the Placement screen, this automation validates the flow cell barcode scanned into the Clarity LIMS by the user using the following logic:

```
if (input.::Flowcell Type:: == ::SP:: && !output.container.name.matches( ::[A-Z0-9]{5}DR[A-Z0-9]{2}:: ) ) {
fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: )
};
if (input.::Flowcell Type:: == ::S1:: && !output.container.name.matches( ::[A-Z0-9]{5}DR[A-Z0-9]{2}:: ) ) {
fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: )
};
if (input.::Flowcell Type:: == ::S2:: && !output.container.name.matches( ::[A-Z0-9]{5}DM[A-Z0-9]{2}:: ) ) {
fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: )
};
if (input.::Flowcell Type:: == ::S4:: && !output.container.name.matches( ::[A-Z0-9]{5}DS[A-Z0-9]{2}:: ) ) {
fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: )
};
```

- Copies the Flowcell Type and Loading Workflow Type field values from step inputs to outputs:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ;
output.::Loading Workflow Type:: = input.::Loading Workflow Type::
```

Validate Run Setup and Generate Sample Sheet Automation

The script definition portion of Validate Run Setup has been moved into the Validation Script custom field due to the 4000 character limitation.

- If modification is required to the Validate Run Setup script, perform the changes directly on Validation Script custom field default value.
- The script definition portion of Generate Sample Sheet is unchanged.

Automatically triggered by selecting a button on the Record Details screen, this automation:

- Copies the Flowcell Type from the step input to the Run Mode field (hidden):

```
step.::Run Mode:: = input.::Flowcell Type::
```

- Validates the parameters entered on the Record Details screen, which is used to set up the run and generate the sample sheet file.
  - Experiment Name only contains alphanumeric, dash, or underscore characters. Spaces are not permitted.

  - When Workflow Type = No Index, both Index Read 1 and Index Read 2 must be 0.
  - When Workflow Type = Single Index, Index Read 1 must be greater than 0 and Index Read 2 must be 0.
  - When Workflow Type = Dual Index, both Index Read 1 and Index Read 2 must be greater than 0.

```
if(!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) {
fail(::Experiment Name contains prohibited characters. Allowed characters are:
a-z, A-Z, 0-9, -, and _::)
};
if (step.::Workflow Type:: == ::No Index::) {
if (step.::Index Read 1:: != 0 || step.::Index Read 2:: != 0) {
fail(::Index Read 1 and Index Read 2 must be 0 if No Index is selected.::)
}
} else if (step.::Workflow Type:: == ::Single Index::) {
if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) {
fail(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if Single
Index is selected.::)
}
} else {
if (step.::Index Read 1:: == 0 || step.::Index Read 2:: == 0) {
fail(::Index Read 1 and Index Read 2 must be greater than 0 if Dual Index is
selected.::)
}
};
```

- Checks the Paired End and Read 2 Cycles field values.
  - When Paired End = True, both Read 1 Cycles and Read 2 Cycles must be greater than 0.
  - When Paired End = False, Read 2 Cycles must be 0.

```
if (step.::Paired End::.toBoolean()) {
   if (step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) {
      fail(::Read 1 Cycles and Read 2 Cycles must be greater than 0 if Paired
      End is True.::)
   }
} else {
   if (step.::Read 2 Cycles:: != 0) {
      fail(::Read 2 Cycles must be 0 if Paired End is False.::)
   }
};
```

- Checks the Flowcell field value.

For Research Use Only. Not for use in diagnostic procedures.

  - If Flowcell Type value is not SP, checks that the value of both Read 1 Cycles and Read 2 Cycles is 151 or less. If the value is greater than 151, an error message displays.

```
if (input.::Flowcell Type::!=::SP:: && step.::Read 1 Cycles:: > 151) {
fail(::Read 1 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
}
if (input.::Flowcell Type::!=::SP:: && step.::Read 2 Cycles:: > 151) {
fail(::Read 2 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
}
```

- Validate Analysis Software Version

```
if (step.hasValue(::Analysis Software Version::) && !step.::Analysis Software
Version::.matches(::^(\\d+)(\\.\\d+)*$::)) {
fail(::Analysis Software Version contains prohibited characters. Allowed
characters are 0-9 and period. It shall start and end with numbers and separated
by single period e.g. 3.8.4::)
};
```

- Checks Override Cycles field value. This field contains only Y, N, I, U, 0–9, and semicolon characters.

```
if (step.hasValue(::Override Cycles::) && !step.::Override Cycles::.matches(::
[YNIU0-9;]+::)) {
fail(::Override Cycles contains prohibited characters. Allowed characters are:
Y, N, I, U, 0-9 and ;. Example: N1Y150;I8;I7N1;Y141U10.::)
};
```

- Sets the next step for samples to ADVANCE, advancing them to the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2) step.

```
nextStep = ::ADVANCE::
```

- Generates the sample sheet and attaches it to the step. For more information, refer to *Sample Sheet Generation* on page 45.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeqDx_BCL2FASTQ_
Samplesheet_v2.csv \
-o {compoundOutputFileLuid0}.csv \
-q true \
-destLIMSID {compoundOutputFileLuid0} \
-l {compoundOutputFileLuid1}
```

Master Step Fields

The following table lists the field configuration details for the fields defined on the Load to Flowcell (NovaSeqDx v1.2) step.

Table 50 Load to Flowcell (NovaSeqDx v1.2) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Analysis Software Version	Text		
BaseSpace Sequence Hub Configuration	Text Dropdown	Required Field	Presets • Not Used • Run Monitoring Only • Run Monitoring and Storage
Custom Recipe Path	Text		
Experiment Name	Text	Required Field	
Index Read 1	Numeric Dropdown	Required Field Custom Entries	Presets • 0 • 6 • 8 Range = 0–20 Decimal places displayed = 0
Index Read 2	Numeric Dropdown	Required Field Custom Entries	Presets • 0 • 6 • 8 Range = 0–20 Decimal places displayed = 0
Library Tube Barcode	Text	Required Field	
Output Folder	Text	Required Field	
Override Cycles	Text		

Field Name	Field Type	Options	Additional Options and Dropdown Items
Paired End	Text Dropdown	Required Field	Presets • True • False
Read 1 Cycles	Numeric Dropdown	Required Field Custom Entries	Presets • 251* • 151 • 101 • 51 Range = 1–251 Decimal places displayed = 0 *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Read 2 Cycles	Numeric Dropdown	Required Field Custom Entries	Presets • 251* • 151 • 101 • 51 Range = 0–251 Decimal places displayed = 0 *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Run Mode (not displayed in user interface)	Text Dropdown	Read Only	Presets • SP • S1 • S2 • S4

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Field Name	Field Type	Options	Additional Options and Dropdown Items
Use Custom Index Read 1 Primer	Toggle Switch		Default • None Set
Use Custom Read 1 Primer	Toggle Switch		Default • None Set
Use Custom Read 2 Primer	Toggle Switch		Default • None Set
Use Custom Recipe	Toggle Switch	Required Field	Default • No
Validation Script	Multiline Text	Required Field Read Only	Default value provided in the drop-down section following the table. Do not remove this field as it is used by Validate Run Setup and Generate Sample Sheet automation script.
Workflow	Text	Read Only	Default • GenerateFASTQ
Workflow Type	Text Dropdown	Required Field	Presets • No Index • Single Index • Dual Index • Custom

Validation Script

```
step.::Run Mode:: = input.::Flowcell Type::; if (!step.::Experiment
Name::.matches(::[a-zA-Z0-9-_]+::)) { fail(::Experiment Name contains prohibited
characters. Allowed characters are: a-z, A-Z, 0-9, -, and _::) }; if
(step.::Workflow Type:: == ::No Index::) { if (step.::Index Read 1:: != 0 ||
step.::Index Read 2:: != 0) { fail(::Index Read 1 and Index Read 2 must be 0 if
```


For Research Use Only. Not for use in diagnostic procedures.

```
No Index is selected.::) } } else if (step.::Workflow Type:: == ::Single
Index::) { if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) { fail
(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if Single
Index is selected.::) } } else { if (step.::Index Read 1:: == 0 || step.::Index
Read 2:: == 0) { fail(::Index Read 1 and Index Read 2 must be greater than 0 if
Dual Index is selected.::) } }; if (step.::Paired End::.toBoolean()) { if
(step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) { fail(::Read 1
Cycles and Read 2 Cycles must be greater than 0 if Paired End is True.::) } }
else { if (step.::Read 2 Cycles:: != 0) { fail(::Read 2 Cycles must be 0 if
Paired End is False.::) }}; if (input.::Flowcell Type:: != ::SP:: && step.::Read
1 Cycles:: > 151) { fail(::Read 1 Cycles must not be larger than 151 if it is
not SPrime Flowcell::) }; if (input.::Flowcell Type:: != ::SP:: && step.::Read 2
Cycles:: > 151) { fail(::Read 2 Cycles must not be larger than 151 if it is not
SPrime Flowcell::) }; if (step.hasValue(::Analysis Software Version::) &&
!step.::Analysis Software Version::.matches(::^(\\d+)(\\.\\d+)*$::)) { fail
(::Analysis Software Version contains prohibited characters. Allowed characters
are 0-9 and period. It shall start and end with numbers and separated by single
period e.g. 3.8.4::) }; if (step.hasValue(::Override Cycles::) &&
!step.::Override Cycles::.matches(::[YNIU0-9;]+::)) { fail(::Override Cycles
contains prohibited characters. Allowed characters are: Y, N, I, U, 0-9 and ;.
Example: N1Y150;I8;I7N1;Y141U10.::) }; step.::Settings Header:: = step.hasValue
(::UMI - Read 1 Length::) || step.hasValue(::UMI - Read 2 Length::) ? ::
[Settings]:: : ::::; nextStep = ::REMOVE::
```

Global Fields

The following table lists the global custom fields that are configured to display on the Load to Flowcell (NovaSeqDx v1.2) step.

Table 51 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Analyte in Derived Sample			
Flowcell Type	Text Dropdown	Required Field	Presets • SP • S1 • S2 • S4

For Research Use Only. Not for use in diagnostic procedures.

Field Name	Field Type	Options	Additional Options and Dropdown Items
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User Interaction

For more information, refer to the Step 3: Load to Flowcell (NovaSeqDx v1.2) section in *NovaSeq 6000Dx Integration v1.2 Validation and Troubleshooting* on page 59.

Protocol 4: AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2)

This final protocol contains one fully automated step, AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2).

This step is fully automated. Do not add samples to the Ice Bucket or start the step manually. If steps are manually started, the sequencing service does not update the samples correctly.

Step 1: AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2)

Step input: Library tube from NovaSeqDx Standard or flow cell from NovaSeqDx Xp protocol

Step output: Result file/measurement

In this step, pooled samples are sequenced on the NovaSeq 6000Dx instrument and the run metrics are recorded in Clarity LIMS.

Set Next Steps Automation

If necessary, override the default next step behavior used by the Sequencer API by performing the following steps:

1. Enable the automation and use it to set custom next step behavior and generate a log file. By default, the automation sets the next step to ADVANCE and the samples complete the protocol.
2. Configure the automation to trigger automatically on exit of the Record Details screen.
3. Disable or configure auto-complete behavior by setting the values of the run.autoComplete and autoCompleteOnlyAtSuccess properties. For details, refer to *Configure autoComplete Properties* on page 57.

Update Lane Number Automation

This automation triggers automatically upon entry to the Record Details screen.

Do not disable or modify this automation to ensure the Lane Number displays properly.

Master Step Fields

The following table shows the master step fields that are configured on the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2) step.

Table 52 Clarity LIMS Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Current Cycle	Numeric	Read Only	Decimal places displayed = 0
Current Read	Numeric	Read Only	Decimal places displayed = 0
Firmware Version	Text	Read Only	
Flow Cell Expiration Date	Date	Read Only	
Flow Cell ID	Text	Read Only	
Flow Cell Lot Number	Text	Read Only	
Flow Cell Mode	Text	Read Only	
Flow Cell Part Number	Text	Read Only	
Flow Cell Side	Text	Read Only	
Instrument Control Software Version	Text	Read Only	
Instrument ID	Text	Read Only	
Instrument Type	Text	Read Only	
Lane Counter	Numeric	Read Only	Default • 1 Hidden
Loading Workflow Type	Text	Read Only	
Output Folder	Text	Read Only	
RTA Version	Text	Read Only	

Field Name	Field Type	Options	Additional Options and Dropdown Items
Run Completion Date	Text	Read Only	Hidden
Run ID	Text	Read Only	
Run Status	Text	Read Only	
Sequencing Log	Multiline Text	Read Only	

Global Fields

The following table shows the global custom fields that capture the run metrics in Clarity LIMS.

Table 53 Custom Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
% Aligned R1	Numeric	Read Only	Decimal places displayed = 2
% Aligned R2	Numeric	Read Only	Decimal places displayed = 2
% Bases >=Q30 R1	Numeric	Read Only	Decimal places displayed = 2
% Bases >=Q30 R2	Numeric	Read Only	Decimal places displayed = 2
% Error Rate R1	Numeric	Read Only	Decimal places displayed = 2
% Error Rate R2	Numeric	Read Only	Decimal places displayed = 2
% Phasing R1	Numeric	Read Only	Decimal places displayed = 3
% Phasing R2	Numeric	Read Only	Decimal places displayed = 3
% Prephasing R1	Numeric	Read Only	Decimal places displayed = 3
% Prephasing R2	Numeric	Read Only	Decimal places displayed = 3

Field Name	Field Type	Options	Additional Options and Dropdown Items
%PF R1	Numeric	Read Only	Decimal places displayed = 2
%PF R2	Numeric	Read Only	Decimal places displayed = 2
Cluster Density (K/mm^2) R1	Numeric	Read Only	
Cluster Density (K/mm^2) R2	Numeric	Read Only	
Intensity Cycle 1 R1	Numeric	Read Only	
Intensity Cycle 1 R2	Numeric	Read Only	
Reads PF (M) R1	Numeric	Read Only	Decimal places displayed = 2
Reads PF (M) R2	Numeric	Read Only	Decimal places displayed = 2
Yield PF (Gb) R1	Numeric	Read Only	Decimal places displayed = 2
Yield PF (Gb) R2	Numeric	Read Only	Decimal places displayed = 2

Note the following details:

- Values are aggregated across all lanes. Some values (eg, Yield PF (Gb) R1) are summed while others are averaged.
- The names listed previously are the default global custom field names installed with the NovaSeqDx v1.2 configuration provided in the Illumina Preset Protocols (IPP) Package v2.6 or later.
- All global configuration fields are configured on the Container entity.
- All field names are configurable via the Clarity LIMS Configuration > Custom Fields screen (Global Fields tab).
- If field names are changed in Clarity LIMS, they must also be changed through the run.metricUdfNames properties file in the `application.yml` file for the Sequencer API. All fields are configured to be visible on the Record Details screen for the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2) step (in the Sample Details table). For more information, refer to the *application.yml Properties* section of *Components Installed* on page 51.

These global custom fields are configured on the Container entity, visible on each input, and must not be confused with per-lane metrics.

All run metrics tracked in Clarity LIMS are overall metrics for the run. There are currently no per-lane metrics provided by NovaSeq 6000Dx Operating Software.

Sample Sheet Generation

The sample sheet is generated on the step before the run. This step is either Dilute and Denature (NovaSeqDx v1.2) in the NovaSeqDx Standard protocol or Load to Flowcell (NovaSeqDx v1.2) in the NovaSeqDx Xp protocol. This step places samples on the library tube or flow cell that is loaded in the NovaSeq 6000Dx instrument.

In the default configuration, the Validate Run Setup and Generate Sample Sheet automation generates one sample sheet file for use with bcl2fastq v2.20 downstream analysis. This file is in the comma-separated values (CSV) format.

The sample sheet is uploaded to the NovaSeq 6000Dx Operating Software (NVOS) via the `/Illumina/Sequencer/v2/sequencing-run/files` endpoint to the Sequencer API. The file endpoint allows for a file to be downloaded from Clarity LIMS using OAuth (instead of Basic Authentication), which is required for NovaSeq 6000Dx.

The run recipe response sets the sample sheet URL to the link to download the file from this endpoint and sets the sampleSheetRequiresOAuth value to true.

Sample Sheet Content

The following step custom fields that display in the Step Details area of the Record Details screen control the sample sheet content:

- Experiment Name
- Read 1 Cycles
- Read 2 Cycles
- Index 1 Cycles
- Index 2 Cycles
- Analysis Software Version
- Override Cycles

For detailed information on the following items, refer to the bcl2fastq2 *Sample Sheet Generation* section of *Illumina Instrument Sample Sheets (NGS v5.17 and later)*.

- Sample sheet generation script parameters and usage
- Sample sheet data and configuration options
- Enabling unique FASTQ file names per sequencing run

NovaSeq 6000Dx Run

The following steps outline the sequence of events that occurs when a flow cell is loaded onto the NovaSeq 6000Dx instrument.

User Authentication and Login

User authentication and login to the NovaSeq 6000Dx instrument is achieved with the getSequencerLoginURL endpoint (`/Illumina/Sequencer/v2/sequencing-run/login`). This endpoint returns the URL that the instrument uses to get an access token.

1. The Sequencer API service provides the NovaSeq 6000Dx Operating Software (NVOS) with a URL for the Clarity LIMS login screen. The NVOS displays this screen to the user. Log in to authorize NVOS to use your account to access Clarity LIMS. This authorization is automatic.
2. Enter credentials:
   - Username and password are passed to the Clarity LIMS server to make sure that the access is valid.
   - By default, the Sequencer API service tries to contact Clarity LIMS at `http://localhost:9080/clarity/`. The installation script sets the URL, but it can be configured with the clarity.url property in the `application.yml` file. For more information, refer to the *application.yml Properties* section of *Components Installed*.
3. The user is redirected to the Sequencer login page at `/Illumina/Sequencer/v2/sequencer_login`.
   - If authentication is successful, the screen displays a success message. The Clarity LIMS server provides the OAuth token and username by redirecting to `/Illumina/Sequencer/v2/sequencer_login/Success`. This redirect does not reload the page, but allows the NovaSeqDx to continue with run setup.
   - If authentication fails, the login screen does not change and a warning message displays. A background redirect to `/Illumina/Sequencer/v2/sequencer_login/Error` informs the NovaSeq 6000Dx of the failure.

Respond to Validation / Recipe Request Call from Instrument

1. The sequencing-run/recipe/NovaSeqDx endpoint consumes a POST request with a LibraryContainerId, FlowCellId, and reagents information.
   - LibraryContainerId, FlowCellId, and reagents are provided by the NovaSeq 6000Dx.
   - LibraryContainerId and FlowCellId can match either the library tube ID or flow cell ID of the corresponding containers in Clarity LIMS.
   - If there are no containers or multiple containers, an error is generated.
2. All populated containers whose name matches either the LibraryContainerId or FlowCellId are validated as follows.

- All samples in the container must be queued for a sequencing run step.
- All samples must be queued for only one sequencing run step.
- All samples must be queued for the same sequencing run step.

> Configure the steps that are considered sequencing run steps in the novaseq.sequenceStepNames property in the `application.yml` file. The property is a list and each entry must be the exact name of a sequencing step, not the name of the underlying master step/process type. For more information, refer to the *application.yml Properties* section of *Components Installed*.

3. After a single container has passed validation, the run setup information (run recipe request) is returned to the NVOS via the API. Then, the run receipt is generated.
   Note the following details:
   - The run recipe information is read from fields configured on the parent process/master step of the samples in the container. The workflow must include recipe definition in that step.
   - By default, the parent process/master step is the Dilute and Denature (NovaSeqDx v1.2) or Load to Flowcell (NovaSeqDx v1.2) step, depending on workflow.
   - The default field values match the names of the fields in the NovaSeq 6000Dx v1.2 configuration included in IPP. The field names are configurable through the recipe.udfNames.* properties. Each property corresponds to an entry in the recipe. The value of a property defines the name of the field that is used to populate that entry in the recipe.
   - It is assumed that this parent process/master step removes any illegal characters from the Experiment Name field.
   - The Clarity LIMS download link to the sample sheet created on the run setup step is used for the SampleSheet value in the recipe. The name of the output of the sample sheet on the setup step is configured through the recipe.sampleSheet.outputName property (Sample Sheet by default) in the `application.yml` file. If no matching output is found, or no file was attached to the output, the value of the recipe.sampleSheet.notAvailableValue property (Not Available by default) is used in the recipe. For more information, refer to the *application.yml Properties* section of *Components Installed*.
4. After the recipe is generated, it is returned as a *.json file in the response, in the form defined by the Swagger definition for the API.

Run Recipe Contents

The sample sheet contents and the fields configured on the Dilute and Denature (NovaSeqDx v1.2) and Load to Flowcell (NovaSeqDx v1.2) steps control the contents of the run recipe.

Example of run recipe content is as follows.

```
{
```

```
"run_name": "NVOS16027_SPrime_2x250",
"run_mode": "S4",
"workflow_type": "DualIndex",
"librarytube_id": "NV0025867-LIB",
"flowcell_id": "",
"sample_loading_type": "NovaSeqStandard",
"rehyb": false,
"paired_end": true,
"read1": 151,
"read2": 151,
"index_read1": 8,
"index_read2": 8,
"output_folder": "\\\\network_path\\run_data",
"samplesheet": "<path to sample sheet on LIMS server>",
"require_samplesheet_authentication": true,
"usecustomrecipe": false,
"customRecipe": null,
"use_basespace": false,
"basespace_mode": null,
"use_custom_read1_primer": false,
"use_custom_read2_primer": false,
"use_custom_index_read1_primer": false
}
```


Respond to Sequencing Started Call from Instrument

1. Matching container for the run (library tube or flow cell) is found, and contents are validated to be queued for the correct step.

   If no matching container is found, or if the samples in the container are incorrectly queued, an error is thrown and the step is not started. This validation is also done during the run recipe request immediately prior, so this error case should not be encountered.

2. Reagent kits that are configured for the Sequencer API are checked against the configuration for the step to be run:
   - If a kit is configured for the API but does not exist in Clarity LIMS, it is created.
   - If a kit exists, but is not enabled on the step to be run, the step configuration is updated to enable it.
     - If a kit that is to be enabled is Archived, it is set to Active.
     - If a kit that is to be enabled is in Pending status, it is set to Active.

- If any kits enabled on the step are not configured for the Sequencer API, a warning is logged, and the API cannot complete the step without user intervention.
- If any kits provided in the recipe request are not configured for the Sequencer API, they are ignored, and a warning is logged during lot tracking.

3. The type of flow cell being used in the run determines the number of replicates for the step.
   - For Standard mode, this information is based on the number of lanes in the flow cell. This information is stored as part of the Sequencer API configuration.
   - For XP mode, there is always one replicate, because there is already one input tracked for each lane.
4. The automated sequencing step is started.
5. Reagent lots are attached to the step. The Flow Cell and Library Tube reagents in the request are ignored. Information from these reagents is tracked in Clarity LIMS as containers and custom fields. Any other reagents in the request are handled as follows.
   - If the reagent is not configured to be tracked, a warning is logged, and the reagent is ignored.
   - You can look up existing reagent lots by kit name (determined by Sequencer API configuration), name (the serial number in the request), and lot number (the lot number in the request).
   - If one or more matching reagent lots exist in Clarity LIMS, the newest one is used.
   - If the expiry date does not match the expiry in the request, or if the status is not set to Active, the lot is updated to have the correct expiry date and an Active status.

   Otherwise a new lot is created with the following configuration:
   - Reagent Kit—Matching kit as determined from Sequencer API configuration and step 2.
   - Name—Serial number in request.
   - Lot Number—Lot number in request.
   - Expiry—Expiry date in request.
   - Status—Active.

   If any reagents are expected to be in the request but are not, these reagents are logged. This action prevents the step from being completed without user intervention.
6. Master step custom fields are updated with the run information parsed from the request. For more information, refer to *Respond to Record InterOps Metrics Call from Instrument* on page 49.
7. Any important events during this request are logged into the Sequencer API log file and in the Sequencing Log multiline global custom field on the step (assuming the step was started successfully). For more information, refer to *Respond to Record InterOps Metrics Call from Instrument* on page 49.

Respond to Record InterOps Metrics Call from Instrument

When the metrics endpoint receives a POST, it completes the following actions:

- Looks for a matching library tube or flow cell in Clarity LIMS.
- Finds the in-progress sequencing step for that container.

When the step is found, the run metrics in the request are written to container global custom fields in Clarity LIMS.

Respond to Sequencing Completed Call from Instrument

If the sequencing run is successful, after the metrics are recorded, the API call for Sequencing Completed is received. The Sequencing Container matches the Information, and the following occurs in Clarity LIMS:

1. The Current Cycle, Current Read, and Status master step custom fields are updated.
2. The multiline Sequencing Log master step field is updated.
3. A success message is recorded in the log file at the info level:

```
Sequencing run with Run ID {runId} of Step '{step.name}' ({step.limsid})
completed successfully.
```

4. Clarity LIMS waits for any automations that are triggered automatically as it advances the step.
5. By default, Clarity LIMS assigns the next steps on the outputs to move them on to the first configured next step. If there are no next steps, the protocol is completed.

Respond to Sequencing Errored Call from Instrument

If the sequencing run is unsuccessful, the API call for Sequencing Error is received. The Sequencing Container matches the Information, and the following occurs in Clarity LIMS:

1. The Current Cycle, Current Read, and Status master step custom fields are updated.
2. The multiline Sequencing Log master step field is updated.
3. A failure message is recorded in the log file at the error level:

```
Step '${Step.name}' (${Step.limsid}) failed with Run ID ${runId}.
```

4. The step is left at the Record Details screen.

Respond to Sequencing RunEndedByUser Call from Sequencer

If the user ends a sequencing run, the API call for Sequencing RunEndedByUser is received. The Sequencing Container matches the Information, and the following occurs in Clarity LIMS:

1. The Current Cycle, Current Read, and Status master fields are updated.
2. The multiline Sequencing Log master step field is updated.
3. The following message is recorded in the log file, at the warning level:

```
"Sequencing run with Run ID ${status.runInfo.runId} of Step
'${step.configuration.name}' (${step.limsid}) aborted by user
${status.runInfo.userName}."
```

4. The step is left at the Record Details screen.

Components Installed

The following sections describe the components (files, properties, reagent categories / label groups, reagent kits, and containers) that are installed by default as part of this integration.

Illumina NovaSeq 6000Dx Integration v1.2 is distributed as an RPM package, BaseSpaceLIMS-sequencer-api. This RPM package must be installed on the Clarity LIMS server.

The BaseSpaceLIMS-sequencer-api RPM installs the following items:

- Sequencer API WAR file
- `application.yml` configuration file
- The following configuration scripts:
  - `configure_sequencer_api_proxy.sh`
  - `configure_sequencer_api_env.sh`
  - `configure_sequencer_api_application.sh`

The NovaSeq 6000Dx v1.2 configuration is delivered through the Illumina Preset Protocols (IPP) Package v2.6. IPP Package v2.6 requires installation of NGS Extensions Package v5.23.0 or later.

- If the NGS Extensions Package is not already installed, or if a version earlier than v5.23.0 is installed, the latest version is installed by default with the NovaSeq 6000Dx integration. For details, refer to *NovaSeq 6000Dx Integration v1.2 Release Notes* on page 89.
- If NGS Extensions Package v5.23.0 or later is already installed, upgrade is not forced or required.

NovaSeq 6000Dx Integration RPM Components

The following table lists the components installed by the RPM package.

Files Installed	Location and Description
`Illumina#Sequencer#v2.war`	`/opt/gls/clarity/tomcat/current/webapps` War file for Sequencer API.
`configure_sequencer_api_application.sh`	`/opt/gls/clarity/config/` Script that configures the Sequencer API application through its external `application.yml` file.
`configure_sequencer_api_env.sh`	`/opt/gls/clarity/config/` Script that configures the Clarity LIMS Tomcat configuration to include Secret Util settings.

Files Installed	Location and Description
`configure_sequencer_api_proxy.sh`	`/opt/gls/clarity/config/` Script that configures the proxy to allow communication with the Sequencer API application.
`sequencer-api.conf`	`/etc/httpd/clarity/` Proxy setting to communicate with Clarity LIMS.

`application.yml` Properties

The `application.yml` file is at `opt/gls/clarity/extensions/sequencing-api/`.

- All properties are configured automatically during installation. You can also configure properties after installation, by editing the `application.yml` file. If you make changes to the file, you must restart Tomcat.
- File properties represent all custom field names, both for recipe and run information.
- For all custom fields, the names (values in this file) can be changed. However, the property names cannot be changed, removed, or added to. For example, the Loading Workflow Type can be changed, but recipe.udfNames.sampleLoadingType cannot.

Table 54 `application.yml` Properties Installed

Property	Description
spring.profiles.active	Tells the application that it is running deployed in Tomcat. Default value: tomcat (Do not change)
clarity.url	Base URL that the Sequencer API service uses to contact the Clarity LIMS. The installation script (configure_ sequencer_api_application) prompts for this property.
clarity.username	Username to be used when communicating with Clarity LIMS.
security.signing-key	Private key that is used when signing/validating OAuth tokens. Changing this property invalidates any issued tokens.
security.token-expiry	Specifies (in hours) the expiry period for login tokens issued by the Sequencer API. Default value: 88

Property	Description
novaseq.sequenceStepNames	List of NovaSeq 6000Dx sequencing run step names for which the integration can find samples queued. • Names must be an exact match to the name of the step in Clarity LIMS (not the master step/process type). • Each line is indented to the same point and start with a dash (-). Default - AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7) - AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2)

novaseq.flowcells	List of supported NovaSeq 6000Dx flow cell types.
	• Type names exactly match the values used by the NVOS run status requests.
	• To add more types, provide both type and lanes properties, indented to the same point and starting with a dash (-).
	Default value:
	`- type: "SP"`
	`lanes: 2`
	`- type: "S1"`
	`lanes: 2`
	`- type: "S2"`
	`lanes: 2`
	`- type: "S4"`
	`lanes: 4`

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Property	Description

novaseq.reagents	List of supported reagent kits to be tracked on sequencing steps.
	- lotName: The name of the lot as it appears in the run started request.
	- kitName: The name of the corresponding reagent kit in Clarity LIMS that is used for lot tracking.
	• The name can differ in the NVOS request versus the Clarity LIMS configuration.
	Default value:
	`- lotName: "Buffer"`
	`kitName: "Buffer Cartridge"`
	`- lotName: "Cluster"`
	`kitName: "Cluster Cartridge"`
	`- lotName: "SBS"`
	`kitName: "SBS Cartridge"`

recipe.udfNames	Configures the names of the fields used in the run recipe. For more information, refer to *Run Recipe Contents* section in *NovaSeq 6000Dx Run* on page 46. • Each value must be enclosed in quotes. Example: recipe.udfNames.runMode "Run Mode" • Value provided for sampleLoadingType must match the value provided in the run configuration. Example: recipe.udfNames.sampleLoadingType "Loading Workflow Type"
recipe.sampleSheet.outputName recipe.sampleSheet.notAvailableValue	Configures the name of the sample sheet file placeholder on the step where run recipe information is populated, and the property value to use when the file is not found.

Property	Description
run.stepUdfNames	Configures the master step field names to be used when recording run results. For more information, refer to the *Master Step Fields* section in *NovaSeq 6000Dx Run* on page 46 • Each value must be enclosed in quotes. Example: run.stepUdfNames.runID "Run ID" • Value provided for sampleLoadingType must match the value provided in the run configuration. • Example: run.stepUdfNames.sampleLoadingType "Loading Workflow Type". It is expected that the run step field and the field on the input derived sample share a name.
run.metricUdfNames	Configures the result file global custom field names to be used when recording run results. These are the fields that capture the run data. For more information, refer to *Global Custom Fields* section.
run.autoComplete	Determines whether the sequencing run step is completed automatically after parsing the run metrics on a run (refer to run.autoCompleteOnlyAtSuccess). Default value: true For more information, refer to the *Configure autoComplete Properties* on page 57 section.
run.autoCompleteOnlyAtSuccess	Determines which of the following options are applicable to the sequencing run step: • Autocompleted when the run status is RunCompletedSuccessfully (if autoCompleteOnlyAtSuccess is true) • Autocompleted regardless of the run status (if autoCompleteOnlyAtSuccess is false). Default value: true For more information, refer to the *Configure autoComplete Properties* on page 57 section.

Reagent Categories/Label Groups

- TruSeq HT Adapters v2 (D7-D5)

Reagent Kits

- Buffer Cartridge
- Cluster Cartridge
- DPX1
- DPX2
- DPX3
- NaOH
- Resuspension Buffer (RSB)
- SBS Cartridge
- Tris-HCl

Container Types

- Library Tube
- SP
- S1
- S2
- S4

This integration supports the following items:

- Library tube with barcode provided in the following formats:
  - RUO mode: [A-Z]{2}[0-9]{7}-[A-Z]{3}
    Example: AB1234567-LIB
  - DX mode: DX[0-9]{7}-[A-Z]{3}
    Example: DX1234567-LIB
- SP, S1, S2, or S4 flow cell with barcode provided in one of the following formats:
  - SP and S1 flow cell: [A-Z0-9]{5}DR[A-Z0-9]{2}
  - S2 flow cell: [A-Z0-9]{5}DM[A-Z0-9]{2}
  - S4 flow cell: [A-Z0-9]{5}DS[A-Z0-9]{2}
    Example: H1991DMXX

NVOS Configuration

For details on configuring the NovaSeq 6000Dx Operating Software (NVOS) for integration with Clarity LIMS, contact the Clarity LIMS Support team.

Routing Script Requirements

The requirements for the routing script functionality are as follows.

- On the steps that use the routing script (Define Run Format (NovaSeqDx v1.2) and Dilute and Denature (NovaSeqDx v1.2)):
  - The Next Step for all samples must be set to Remove from workflow. A script sets this value. The value must not change in the Assign Next Steps screen.
- In the protocol configuration screen, the following settings are required and must not be changed:
  - In the Next Steps section, for the last step in the protocol, the method of assigning the next step must be set to Automatic.

Configure autoComplete Properties

The `application.yml` configuration file contains the following properties:

- run.autoCompleteOnlyAtSuccess
- run.autoComplete

These properties determine the conditions under which the last step of the NovaSeq 6000Dx workflow, AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2), is automatically completed. The two scenarios for autocompletion of the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2) step are as follows.

- The step autocompletes only when the run status is RunCompletedSuccesfully (default behavior).
- The step autocompletes regardless of the run status.

By default, both properties are set to true and the step only completes if the run is successful. If the run fails or is aborted, the step must be completed manually in Clarity LIMS.

For the step to autocomplete regardless of the result of the sequencing run, change the autoCompleteOnlyAtSuccess property value to false.

Enable/Disable autoComplete Properties

1. Open the `application.yml` file at `/opt/gls/clarity/extensions/sequencer-api/application.yml`:

```
vi /opt/gls/clarity/extensions/sequencer-api/application.yml
```

2. Edit the value of the autoCompleteOnlyAtSuccess property as required.
3. Save the file.

autoComplete Properties

The following table shows how the combined value of the run.autoComplete and run.autoCompleteOnlyAtSuccess properties affects the autoComplete behavior of the sequencing step.

Table 55 run.autoComplete and run.autoCompleteOnlyAtSuccess Value Matrix

run.autoComplete property value	run.autoCompleteOnlyAtSuccess property value	Outcome
true	true	• Step automatically completes only if the sequencing run is successful. • If run is not successful, the step does not automatically complete, and the run details are recorded in Clarity LIMS.
true	false	• Step automatically completes, regardless of the run status.
false	true	• Step does not automatically complete. • Run details are recorded in Clarity LIMS.
false	false	

Rules and Constraints

- The workflow configuration contains several validation checks. To make sure that the calculations work properly, it is important that you do not disable any of this validation logic. The validation checks determine the following information:
  - Which samples, and how many, can enter each step together.
  - Which samples, and how many, can be pooled together.
- The library tube ID must be unique. There must not be multiple library tube containers in the system with the same name.
- Reagent labels (indexes) must be unique.
- Only controls are permitted as unindexed samples. All other unindexed samples and pools are not permitted.
- For sample sheet generation constraints, refer to the *Bcl2fastq2 Sample Sheet Generation* section in *Illumina Instrument Sample Sheets (NGS v5.17 & later)*.
- Do not manually start the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2) step. This step is fully automated and the sequencing service does not update samples correctly if they have been manually started.
- For the automated run to start successfully, the Validate Run Setup and Generate Sample Sheet button must be selected.

NovaSeq 6000Dx Integration v1.2 Validation and Troubleshooting

This section explains how to validate the installation of the Illumina NovaSeqDx Integration Package v1.2.

The validation process involves the following items:

- Running samples through the Library Prep Validation workflow.
  - The workflow contains a single-step protocol that models the library prep required to produce normalized libraries. At the end of the step, the normalized libraries advance to the workflow selected.
- Running normalized libraries through the NovaSeqDx v1.2 workflow validates the following items:
  - Successful routing of samples from the Run Format (NovaSeqDx v1.2) step to the NovaSeqDx Standard (NovaSeqDx v1.2) or NovaSeqDx Xp (NovaSeqDx v1.2) step.
  - Automated generation of v2 sample sheet. This file automatically uploads to the sequencing system via the Sequencer API.
  - Automatic validation of run setup information. Information is uploaded to the NovaSeq 6000Dx Operating Software (NVOS) via the Sequencer API and is used to create the run recipe and initiate the run.
  - Automated tracking of the NovaSeq 6000Dx sequencing run and parsing of run statistics into Clarity LIMS, via the Sequencer API.

The validation steps assume that the NovaSeqDx Integration Package v1.2 is installed and you have imported the default Clarity LIMS configuration.

Activate Workflow, Create Project, Add and Assign Samples

The following steps set up Clarity LIMS in preparation for running samples through the Library Prep Validation v2.3.1 and NovaSeqDx v1.2 workflows.

1. On the Configuration tab, under Workflows, activate both the Library Prep Validation v2.3.1 and NovaSeqDx v1.2 workflows.
2. On the Projects and Samples screen, create a project and add samples to it.
3. Assign the samples to the Library Prep Validation workflow.

Library Prep Protocol: Library Prep Validation v2.3.1

This single-step protocol models the library prep required to produce normalized libraries that are ready for the NovaSeqDx v1.2 workflow.

Run Library Prep Validation v2.3.1

1. In Lab View, locate the Library Prep Validation v2.3.1 protocol.
   Samples are queued for the Library Prep Validation v2.3.1 step.

2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Placement screen, complete the following actions:
   a. In the Placed Samples area on the right, in the Container field, scan or enter the barcode of the destination container.
   b. Select the samples from the Samples to be Placed area on the left, and drag them to the container wells on the right.
   c. Select Add Labels.
5. On the Add Labels screen, the Labels to Add list is populated with labels from the TruSeq HT Adapters v2 (D7-D5) label group. The group is the only label group configured in the integration and is used by default in this step.
   a. Select labels on the left, and drag them to the container on the right to assign them to placed samples.
   b. Select Record Details.
6. On the Record Details screen, complete the following actions:
   a. In the Sample Details table, enter values for Normalized Molarity (nM). Type values individually or copy and paste to enter multiple values from a spreadsheet.
   b. The Sequencing Instrument column indicates the instrument to be used for the sequencing run. Select NovaSeqDx.
   c. Populate the fields in this column using one of the following methods:
      - To populate fields individually for each sample, select from the drop-down list in each row.
      - To populate all fields at one time, copy and paste from a spreadsheet.

   Alternatively, select inside the table and press Ctrl + A to select all rows. At the top of the table, select Sequencing Instrument and then select NovaSeqDx from the adjacent drop-down list. Select Apply.

   > If working with many samples, copy and paste to insert multiple values from a spreadsheet as follows.
   >
   > a. Select Copy Samples to copy the sample information from Clarity LIMS to the Clipboard.
   > b. Open a blank Excel document and paste the contents of the clipboard into the spreadsheet.
   > c. In the spreadsheet, enter the Molarity (nM) and Sequencing Instrument values. Select the entire spreadsheet including the headers (Ctrl + A), and copy (Ctrl + C) the information to the Clipboard.
   > d. Within Clarity LIMS, select the Paste Samples button. Paste the contents of the clipboard (Ctrl + V) into the Update Table window and then select Update to paste the contents into the Sample Details table.
7. Select Next Steps.

Triggers the Set Next Steps automation, which sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step.

8. On the Assign Next Steps screen in the Samples table, the Next Step value for all samples is prepopulated with Remove from workflow.

   Do not change this value. If Next Step is not set to Remove from workflow, the routing script does not route samples correctly.

9. Select Finish Step.
   On exit from the step, the Routing Script automation is triggered. This automation assigns samples to the first step of the NovaSeqDx v1.2 workflow, Define Run Format (NovaSeqDx v1.2). This is the only step in Protocol 1: Run Format (NovaSeqDx v1.2).

Protocol 1: Run Format (NovaSeqDx v1.2)

This protocol includes a single step, Define Run Format (NovaSeqDx v1.2). The step allows for the assignment of per sample values for the following fields:

- NovaSeqDx Run Mode
- Loading Workflow Type
- Normalized Molarity
- Flowcell Type
- Final Loading Concentration (pM)

At the end of the step, samples are routed to the NovaSeqDx Standard or NovaSeqDx Xp protocol. This routing is done according to the selected Loading Workflow Type. The selected NovaSeqDx Run Mode must be compatible with the Loading Workflow Type and Flowcell Type. The following table shows the compatibility for the combinations of each Flowcell Type based on the NovaSeqDx Standard protocol.

Table 56 NovaSeqDx Standard Protocol Compatibility

Flowcell Type	Run Mode
SP	RUO
S1	RUO
S2	RUO, DX
S4	RUO, DX

The following table shows the compatibility for the combinations of each Flowcell Type based on the NovaSeqDx Xp protocol.

Table 57 NovaSeqDx Xp Protocol Compatibility

Flowcell Type	Run Mode
SP	RUO
S1	RUO
S2	RUO
S4	RUO

Step 1: Define Run Format (NovaSeqDx v1.2)

1. In Lab View, locate the Run Format (NovaSeqDx v1.2) protocol. The samples are queued for the Define Run Format (NovaSeqDx v1.2) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Record Details screen in the Sample Details table, populate the following fields (values can vary across samples):
   - NovaSeqDx Run Mode—Select DX or RUO.
   - Loading Workflow Type—Select NovaSeq Standard or NovaSeq Xp from the drop-down list.
   - Flowcell Type—Select SP, S1, S2, or S4.
   - Final Loading Concentration (pM)—Select from the two preset options: 225 (for PCR-free workflows), 400 (for Nano workflows), or enter a different value.
   - Normalized Molarity (nM)—These values are copied over from the previous step. If this column is not populated during library prep, enter the values here.
5. Select Next Steps, which triggers the Set Next Steps automation and the following actions:
   - Sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step.
   - Checks that the NovaSeqDx Run Mode value is compatible with the Loading Workflow Type and Flowcell Type. This automation also generates an error message if the Loading Workflow Type or the Flowcell Type are incompatible.
   - Calculates the Minimum Molarity.
   - Checks Normalized Molarity value. For samples with no Normalized Molarity value (eg, empty value, not including 0), generates an error message notifying that the field cannot be empty.
   - Compares each sample Normalized Molarity value with the Minimum Molarity value.
6. On the Assign Next Steps screen:
   - In the Sample Details table, the Next Step for all samples is prepopulated with Remove from workflow, regardless of the Loading Workflow Type.

> Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not routed samples correctly.

- For samples whose Normalized Molarity value is lower than the Minimum Molarity value, the Loading Workflow Type is set to Remove from workflow. A message is recorded in the Warning field for the sample.
  To proceed, choose one of the following options:
  - Return to the Record Details screen and adjust the Normalized Molarity value so that it equals or exceeds the Minimum Molarity value. The Loading Workflow Type must be set to NovaSeq Standard or NovaSeq Xp, as applicable.
  - Complete the protocol without correcting the Normalized Molarity value. In this case, the samples in question are removed from the Clarity LIMS workflow.

7. Select Finish Step. The Routing Script automation is triggered:
   - Samples whose Loading Workflow Type is set to Remove from workflow are removed from the Clarity LIMS workflow. (Occurs when the Normalized Molarity value is lower than the Minimum Molarity.)
   - Samples whose Loading Workflow Type is set to NovaSeq Standard are routed to the Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.2) step. This step is the first step in the NovaSeqDx Standard protocol.
   - Samples whose Loading Workflow Type is set to NovaSeq Xp are routed to the Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.2) step. This step is the first step in the NovaSeqDx Xp protocol.

Protocol 2: NovaSeqDx Standard (NovaSeqDx v1.2)

In this protocol, samples are pooled and added to the library tube in preparation for the NovaSeq 6000Dx run. The protocol contains two steps:

1. Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.2)
2. Dilute and Denature (NovaSeqDx v1.2)

Step 1: Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.2)

1. In Lab View, locate the NovaSeqDx Standard (NovaSeqDx v1.2) protocol.
   Samples for the Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.2) step are queued.
2. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
   When the step begins, the Validate Single NovaSeqDx Run Mode automation is triggered. This automation checks that the samples have the same NovaSeqDx Run Mode (RUO or DX).
4. On the Pooling screen, perform the following actions:

a. Create a pool by dragging samples into the Pool Creator.

> Only create one pool.

b. Enter a name for the pool or accept the default name (eg, Pool #1).

c. Select Record Details.
After exiting the Pooling screen, the Validate Inputs Flowcell Type and Single Pool is triggered. The automation verifies the following items:

- All samples in the pool have been assigned the same Flowcell Type.
- Only one pool has been created.

5. On the Record Details screen in the Step Details area, complete the two required fields and one optional field, as needed:

- Number of Flowcells to Sequence—Used in volume calculations, to make sure that the volumes are sufficient for the number of times the pool is sequenced.
- Minimum Per Sample Volume (µl)—Used to calculate how much of each sample is included in the pool. The field is prepopulated with the configured default value 5 µl, but can be edited.
- % PhiX (2.5 nM) Spike-In—Used to calculate the volume of PhiX v3 control included in the pool for the given percentage of spike-in. The field is optional and can be edited.

> Assuming the default Minimum Per Sample Volume (µl) value of 5, for a given batch:
>
> - If the smallest Per Sample Volume (µl) value is < 5, Clarity LIMS automatically assigns a value of 5 to the Adjusted Per Sample Volume (µl) field.
> - Clarity LIMS changes the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

6. In the Sample Details table, select the pool icon to view details on the pool composition.
7. Select Calculate Volumes to trigger the Calculate Volumes automation. This automation performs calculations based on the selected Flowcell Type.

- Calculates Bulk Pool Volume (µl) value.
- Sets the value of the Bulk Pool Volume (µl) field.
- Calculates the PhiX Volume (µl) value if % PhiX (2.5 nM) Spike-In has value.
- Calculates the Per Sample Volume (µl) value to be added to the pool.
- Calculates the Total Sample Volume (µl) value.
- If the Total Sample Volume is less than the Bulk Pool Volume, it calculates the RSB Volume (µl) value.
- Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
- Populates the Volume of Pool to Denature (µl), NaOH Volume (µl) and Tris-HCl Volume (µl) columns of the Sample Details table. The script sets the values, and is not editable.

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- The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains volume information about the pool and the individual samples that it contains. Select the file to download it and then open it in Excel.

8. Select Next Steps to trigger the Set Next Step automation. This automation sets the next step for samples to ADVANCE and advances them to the next step in the protocol, Dilute, and Denature (NovaSeqDx v1.2). The automation also copies the NovaSeqDx Run Mode value from the input pool to the output pool.
   On the Assign Next Steps screen, the next step for samples is set to the next step in the workflow: Dilute and Denature (NovaSeqDx v1.2).
9. Select Finish Step.
   At the end of this step, the pool of samples automatically advances to the Dilute and Denature (NovaSeqDx v1.2) step.

Step 2: Dilute and Denature (NovaSeqDx v1.2)

1. In Lab View, locate the NovaSeqDx Standard (NovaSeqDx v1.2) protocol. The pool of samples queued for the Dilute and Denature (NovaSeqDx v1.2) step are listed.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work. The Validate Single Input automation is triggered. This automation verifies that only one container is input for the step.
4. On the Placement screen:
   a. Drag the pool into the library tube in the Placed Samples area.
   b. Scan or type the barcode of the library tube into the Library Tube field.
   c. Select Record Details.
   After exiting the Placement screen, the Validate Library Tube Barcode automation makes sure that the library tube barcode conforms to the barcode mask that corresponds with the NovaSeqDx Run Mode in the following table. If the barcode does not match, an error message displays. The automation also copies the Flowcell Type and Loading Workflow Type values from step inputs to outputs.

NovaSeqDx Run Mode	Valid Barcode Mask
RUO	[A-Z]{2}[0-9]{7}-[A-Z]{3}
DX	DX[0-9]{7}-[A-Z]{3}

5. On the Record Details screen, the Reagent Lot Tracking section lets you tracks the NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. Add and activate lots for these reagents as follows.
   a. In a new browser tab, open an additional instance of Clarity LIMS.
   b. Navigate to the Consumables configuration screen.

c. Select the NaOH reagent kit and select New Lot.
d. Enter the lot details.
e. Set the Status of Reagent Lot toggle switch to Active.
f. Select Save.
g. Repeat this process to add and activate Resuspension Buffer and Tris-HCl reagent lots.
h. Return to your original working browser tab and refresh the page.

6. In the Reagent Lot Tracking section, select from the active lots displayed in each drop-down list. The fields displayed on the Record Details screen are used to set up the run and generate the sample sheet. Some of these fields are autopopulated and some must be completed manually.

Table 58 Fields Displayed on Record Details Screen of Dilute and Denature (NovaSeqDx v1.2)

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.
BaseSpace Sequence Hub Configuration	Presets • Not Used • Run Monitoring Only • Run Monitoring and Storage
Workflow	Automatically populated with the preset GenerateFASTQ.
Workflow Type	Presets • No Index • Single Index • Dual Index • Custom
Index Read 1	Presets • 0 • 6 • 8 • Type a value between 0 and 20
Index Read 2	Presets • 0 • 6 • 8 • Type a value between 0 and 20
Paired End	Presets • True • False

Field	Value
Read 1 Cycles	Presets • 251 • 151 • 101 • 51 • Type a value between 1 and 251 *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Read 2 Cycles	Presets • 251 • 151 • 101 • 51 • Type a value between 0 and 251 *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Use Custom Read 1 Primer	Select if applicable.
Use Custom Read 2 Primer	Select if applicable.
Use Custom Index Read 1 Primer	Select if applicable.
Use Custom Recipe	Select if applicable.
Custom Recipe Path	If the Use Custom Recipe option is selected, enter the path to the custom recipe file to be used.
Output Folder	Enter network path for sequencing run folder. For example: `\\networkshare\run_data`
Analysis Software Version	Enter the software version for BCL Convert Analysis for v2 sample sheet. Leave blank if not applicable.
Override Cycles	Enter the Override Cycles value for v2 sample sheet. Leave blank if not applicable.

7. On the Record Details screen, select Validate Run Setup and Generate Sample Sheet. This triggers the automation script, which performs the following actions:
   - Validates the parameters entered on the Record Details screen.
   - Generates the v2 sample sheet and attaches it to the placeholder in the Files area of the Record Details screen.

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> The v2 sample sheet generated does not include Cloud and Analysis sections. Contact Illumina Support for information on generating v2 sample sheets with those sections.

- Select Next Steps.

8. On the Assign Next Steps screen, the Next Step for samples is prepopulated with Remove from workflow. The Routing Script automation expects this value, and requires the value to advance samples to the next step.

   > Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.

9. Select Finish Step.
   After exiting the step, the following actions occur:
   - The Routing Script automation is triggered and samples are routed to AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2).
   - In Lab View, the pool of samples is queued for the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2) step.

   At this point in the workflow, the user interaction ends. Proceed to AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2).

   > Do not add samples to the Ice Bucket or start the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2) step. The integration adds samples automatically.

Protocol 3: NovaSeqDx Xp (NovaSeqDx v1.2)

In this protocol, samples are pooled and added to lanes on the NovaSeq 6000Dx flow cell. The option selected in the Define Run Format (NovaSeqDx v1.2) step determines the flow cell type.

The protocol contains three steps:

1. Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.2)
2. Dilute, Denature & ExAmp (NovaSeqDx v1.2)
3. Load to Flowcell (NovaSeqDx v1.2)

Step 1: Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.2)

1. In Lab View, locate the NovaSeqDx Xp (NovaSeqDx v1.2) protocol. The samples are queued for the Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.2) step.
2. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Pooling screen, do as follows.
   a. Create a pool by dragging samples into the Pool Creator.

      > Create only one pool.

b. Enter a name for the pool or accept the default name (eg, Pool #1).

c. Select Record Details.

5. On exiting the Pooling screen, the Validate Inputs Flowcell Type and Single Pool automation is triggered. The automation verifies the following items:
   - All samples in the pool are assigned the same Flowcell Type.
   - Only one pool was created.
6. On the Record Details screen, in the Step Details area, complete the two required fields and one optional field, as needed:
   - Number of Lanes to Sequence—Used in volume calculations to make sure that volumes are sufficient for the number of times the pool is sequenced.
   - Minimum Per Sample Volume (µl)—Used to calculate how much of each sample is included in the pool. The field is prepopulated with the configured default value 5 µl, but can be edited.
   - % PhiX (0.25nM) Spike-In—Used to calculate the volume of PhiX v3 control to be included in the pool for the given percentage of spike-in. The field is optional and can be edited.

     Assuming the default Minimum Per Sample Volume (µl) value of 5, for a given batch:

     - If the smallest Per Sample Volume (µl) value is < 5, Clarity LIMS automatically assigns a value of 5 to the samples Adjusted Per Sample Volume (µl) field.
     - Clarity LIMS then adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.
7. [Optional] In the Sample Details table, select the pool icon to view details on the pool composition.
8. Select Calculate Volumes to trigger the Calculate Volumes automation. This automation performs volume calculations based on the selected Flowcell Type, as follows.
   - Calculates Bulk Pool Volume (µl) value.
   - Calculates the Per Sample Volume (µl) value to be added to the pool.
   - Calculates PhiX Volume (µl) value if % PhiX (0.25nM) Spike-In has value.
   - Calculates the Total Sample Volume (µl) value.
   - If the Total Sample Volume is less than the Bulk Pool Volume, the automation calculates the RSB Volume (µl) value.
   - Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
   - Generates the Calculation File (CSV) and attaches it to the step. This file contains volume information about the samples and RSB buffer to add to the pool. Select the file to download it, and then open it in Excel.
9. Select Next Steps to trigger the Set Next Step automation. This automation performs the following actions:

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- Copies the Flowcell Type values from the step inputs to the step outputs.
- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol—Dilute, Denature & ExAmp (NovaSeqDx v1.2).

On the Assign Next Steps screen, the next step for samples is set to Dilute, Denature & ExAmp (NovaSeqDx v1.2).

10. Select Finish Step.
    At the end of this step, the pool of samples automatically advances to the Dilute, Denature & ExAmp (NovaSeqDx v1.2) step.

Step 2: Dilute, Denature & ExAmp (NovaSeqDx v1.2)

1. In Lab View, locate the NovaSeqDx Xp (NovaSeqDx v1.2) protocol. The pool of samples queued for the Dilute, Denature & ExAmp (NovaSeqDx v1.2) step displays.
2. Add the pool to the Ice Bucket and select View Ice Bucket.
3. [Optional] On the Ice Bucket screen, set the number of derivatives to create (placed into the flow cell lanes) and select Begin Work.
4. On entry to the Record Details screen, the Calculate Volumes automation is triggered.
   This automation sets the following values based on the selected Flowcell Type:
   - BP Aliquot Volume (µl)
   - NaOH Volume (µl)
   - Tris-HCl Volume (µl)
   - DPX1 Volume (µl)
   - DPX2 Volume (µl)
   - DPX3 Volume (µl)
   - Mastermix per Lane (µl)
   - The automation also populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
   - The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains information about the DPX Mastermix volume and the volume of Mastermix, NaOH, and Tris-HCl to add per working pool (refer to the next step).
5. On the Record Details screen, the Reagent Lot Tracking section tracks the DPX1, DPX2, DPX3, NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. These reagents must be added.
   To add and activate reagent lots, do as follows.
   a. In a new browser tab, open an additional instance of Clarity LIMS.
   b. From Configuration, select the Consumables tab.

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c. From Consumables, select Reagents.
d. Select one of the reagent kits previously listed and select New Lot.
e. Enter the lot details.
f. Set the Status of Reagent Lot toggle switch to Active.
g. Select Save.
h. Repeat this process to add and activate the remaining reagent lots.
i. Return to your original working browser tab and refresh the page.

6. On the Record Details screen, perform the following actions:
   a. In the Reagent Lot Tracking section, select from the active lots displayed in each drop-down list.
   b. In the Step Details section, verify that the DPX1, DPX2, and DPX3 reagent volume values are populated. The script sets these values and they cannot be edited.
   c. [Optional] In the Sample Details table, select the pool icon to view details of the working pool composition.
   d. Make sure that the following values and columns are populated:
      - BP Aliquot
      - Mastermix per lane
      - NaOH
      - Tris-HCl
      - Flowcell Type
      - Loading Workflow Type

      A script sets these values and columns and they cannot be edited. The working pool number is appended to the bulk pool name, so that you can identify which working pools are derived from the same bulk pool.
7. In the Files area, select the Calculation File (CSV) to open it and view details on the DPX Mastermix volume and the volume of Mastermix, NaOH, and Tris-HCl to add per working pool.
8. Select Next Steps.
   On the Assign Next Steps screen, the next step is already set to Load to Flowcell (NovaSeqDx v1.2).
9. Select Finish Step.

Step 3: Load to Flowcell (NovaSeqDx v1.2)

1. On the Ice Bucket screen, perform the following actions:
   a. In the Container Options panel, select the appropriate flow cell type from the Destination Container drop-down list.
   b. Select Begin Work.

2. The Validate Inputs and Selected Container automation checks the following information:
   - The Flowcell Type field is set to a valid value (SP, S1, S2, or S4) and each input has the same value for the field.
   - The container type selected matches the value in the Flowcell Type field.
   - The number of outputs matches the number of lanes on the selected flow cell type. If validation fails, an error message reports the number of working pools does not match the number of lanes available on the flow cell.
3. On the Placement screen, do as follows.
   a. Drag pools from the left of the screen over into the Placed Samples area on the right.
   b. Scan or type the barcode of the flow cell into the Flowcell field.
   c. Select Record Details.

   After exiting the Placement screen, the Validate Flowcell Barcode automation makes sure that the container barcode conforms to the barcode mask for the selected Flowcell Type. This automation also copies the Flowcell Type and Loading Workflow Type field values from step inputs to outputs. The following table shows the barcode mask information.

Flowcell Type	Barcode Mask
SP	[A-Z0-9]{5}DR[A-Z0-9]{2}
S1	[A-Z0-9]{5}DR[A-Z0-9]{2}
S2	[A-Z0-9]{5}DM[A-Z0-9]{2}
S4	[A-Z0-9]{5}DS[A-Z0-9]{2}

4. The fields displayed on the Record Details screen are used to set up the run and generate the sample sheet.
   Some of these fields are autopopulated and some must be entered manually. Refer to the following table for details.

   Table 59 Fields Displayed on Record Details Screen of Load to Flowcell (NovaSeqDx v1.2) Step

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. No spaces.
BaseSpace Sequence Hub Configuration	Presets • Not Used • Run Monitoring Only • Run Monitoring and Storage

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Field	Value
Workflow	Presets • GenerateFASTQ
Workflow Type	Presets • No Index • Single Index • Dual Index • Custom
Index Read 1	Presets • 0 • 6 • 8 • Type a value between 0 and 20
Index Read 2	Presets • 0 • 6 • 8 • Type a value between 0 and 20
Paired End	Presets • True • False
Read 1 Cycles	Presets • 251* • 151 • 101 • 51 • Type a value between 1 and 251 *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Read 2 Cycles	Presets • 251* • 151 • 101 • 51 • Type a value between 0 and 251 *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Use Custom Read 1 Primer	Select if applicable.

Field	Value
Use Custom Read 2 Primer	Select if applicable.
Use Custom Index Read 1 Primer	Select if applicable.
Use Custom Recipe	Select if applicable.
Custom Recipe Path	If you selected the Use Custom Recipe option, enter the path to the custom recipe file to be used.
Output Folder	Enter network path for sequencing run folder. For example: `\\networkshare\run_data`
Library Tube Barcode	Scan the library tube barcode.
Analysis Software Version	Enter the software version for BCL Convert Analysis for v2 sample sheet. Leave blank if not applicable.
Override Cycles	Enter the Override Cycles value for v2 sample sheet. Leave blank if not applicable.

5. Select Validate Run Setup and Generate Sample Sheet to trigger the automation script. The script performs the following actions:
   - Validates the parameters entered on the Record Details screen.
   - Generates the sample sheet and attaches it to the placeholder in the Files area on the Record Details screen.

   The v2 sample sheet generated does not include Cloud and Analysis sections. Contact Illumina Support for information on generating v2 sample sheets with these sections.
6. Select Next Steps.
   On the Assign Next Steps screen, the Next Step field for samples is prepopulated with Mark protocol as complete.
7. Select Finish Step.
   At this point in the workflow, the user interaction ends. The flow cell is queued for the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2) step.

Proceed to AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2).

Do not add samples to the Ice Bucket or start the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2) step. The integration adds samples automatically.

Protocol 4: AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2)

This protocol contains a single fully automated step: AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2).

The integration starts the step automatically and data from the run is parsed back into Clarity LIMS. User interaction is not required, but you can review the stages of the step in Clarity LIMS (refer to Review Run Data).

Automations

The NovaSeqDx Run (NovaSeqDx v1.2) step contains two automations:

- Set Next Steps—This automation is disabled (Set to Not Used). By default, the Sequencer API is used to set the next step for output samples.
  The Illumina Preset Protocols (IPP) Package include a Set Next Steps automation configured on the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2) step. You can use this automation to override the default next step behavior. For more information, refer to *NovaSeq 6000Dx Integration v1.2 Configuration and User Interaction* on page 1.
- Update Lane Number—This automation is triggered automatically upon entry to the Record Details screen. To make sure that the Lane Number displays correctly, do not disable or modify this automation.

Review Run Data

Read summary metrics are recorded for the library pool. After the run is complete, open the step and review these metrics displayed on the Next Steps screen in the Step Data section and the Sample Details table.

Step Data Section

The following values populate the master step fields:

- Run ID
- Run Status
- Output Folder
- Current Cycle
- Current Read
- Loading Workflow Type
- Flow Cell ID
- Flow Cell Side
- Flow Cell Mode
- Flow Cell Part Number
- Flow Cell Lot Number
- Flow Cell Expiration Date
- Instrument ID

- Instrument Type
- Instrument Control Software Version
- Firmware Version
- RTA Version
- Sequencing Log

Sample Details Table

Summary metrics populate the global custom fields listed. Values are aggregated across all lanes. Some values (eg, Yield PF (Gb) R1) are summed, while others are averaged.

- Yield PF (Gb) R1
- Yield PF (Gb) R2
- Reads PF (M) R1
- Reads PF (M) R2
- Cluster Density (K/mm^2) R1
- Cluster Density (K/mm^2) R2
- %PF R1
- %PF R2
- % Bases >=Q30 R1
- % Bases >=Q30 R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- % Aligned R1
- % Aligned R2
- % Error Rate R1
- % Error Rate R2

How the Integration Works

The following steps summarize how the Sequencer API integration works. For details on sample sheet generation and the sequence of events involved in the NovaSeq run, refer to *NovaSeq 6000Dx Integration v1.2 Configuration and User Interaction* on page 1.

1. When setting up a run on the NovaSeq 6000Dx instrument, NVOS sends a request for the run recipe.
   a. The Sequencer API validates that samples and containers are correctly queued for the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2) step.
   b. Clarity LIMS sends a JSON response to NVOS. The response includes the run recipe information and a link to download the sample sheet (used with analysis software such as bcl2fastq2).
2. When the run starts, NVOS sends a RunStarted run status request. The Sequencer API then does the following:
   a. Validates reagent kit information in the RunStarted request:
      - Verifies that the reagent kit exists. If the reagent kit does not exist, the Sequencer API creates the kit and enables on the master step.
      - Verifies that the reagent kit is activated. If the reagent kit is not active, the Sequencer API activates it.
   b. Initiates the step for the queued samples. The step produces one output file placeholder per lane of the flow cell in use, based on the flow cell type.
   c. Records all relevant information from the RunStarted request on the step (such as reagent lots and step fields).
3. At the end of the run, NVOS sends one of the following run status requests:
   - RunCompletedSuccessfully
   - RunEndedByUSer
   - RunErroredOut

   The Sequencer API updates the step with any new information, which includes only the status and cycle/read information.
4. After primary analysis completes, NVOS sends a request containing the parsed run metrics. NVOS sends a request only when the run completes successfully. However, the API does not make this assumption and accepts the request regardless of status. The Sequencer API then does the following:
   a. Records the metrics into the fields on file placeholder outputs in Clarity LIMS.
   b. Completes the step in Clarity LIMS if the status is RunCompletedSuccessfully. For all other status options, the step remains in progress. This action is the default autocomplete step behavior. For more information, refer to *NovaSeq 6000Dx Integration v1.2 Configuration and User Interaction* on page 1.

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NovaSeq 6000Dx Integration v1.2 Configuration and User Interaction on page 1 provides detailed information on how the following components work with the Sequencer API integration:

- Sample sheet generation and contents
- NovaSeq 6000Dx run
- User authentication and login
- Run recipe contents

Troubleshooting

If an automation trigger does not appear to run its corresponding scripts, refer to the following sections in the Clarity LIMS API documentation:

- *Troubleshooting Automated Worker*
- *Troubleshooting Automation* in the API

If an error occurs that does not provide direction on how to proceed, (eg, the error is related to the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2) step) review the log file information. For more details, refer to *Logging* on page 78.

If the automated run step starts, but does not complete, do as follows.

1. Use one of the following methods to open the in progress step in Clarity LIMS:
   - Log in to the default user account.
   - In Lab View, find the step in the Recent Activities pane.
   - Search for the step in Clarity LIMS. You can use the Library Tube or Flowcell barcode as the search term. The steps depend on whether the search is a NovaSeqDx Standard or NovaSeqDx Xp run.
2. On the Record Details screen, the Sequencing Log multiline text field contains logging information. If unable to reach the Record Details screen, or if the Sequencing Log field does not contain enough information to resolve the issue, review the `sequencer-api.log` file. For details, refer to *Logging* on page 78.
3. Contact the Clarity LIMS support team. Provide the relevant information from the troubleshooting steps already performed.

Logging

In addition to updating the Sequencing Log multiline text field on the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2) step, the Sequencer API writes a detailed log file to `/opt/gls/clarity/tomcat/current/logs/sequencer-api.log`.

Log messages include the Library Tube ID and Flow Cell ID whenever the messages are related to a sequencing run-related request. In the Clarity LIMS workflows, these IDs are recorded as container names.

Some log messages are not directly related to a sequencing run request, such as downloading the sample sheet. In this case, the file LIMS ID and file name are included in log messages. The sample sheet downloaded by the sequencing system has a name that includes the sequencing container ID (Library Tube for Standard or Flowcell for Xp). The relevant container name search finds most of the log messages for this request. However, the LIMS ID of the file must be known in order to retrieve all messages related to the file download. The LIMS ID is found in the Clarity LIMS API and, for sample sheets used in a run, in the recipe response (which can also be found in the log file).

Example lines from the log file:

```
2021-06-19 21:32:19.705 INFO --- [] SequencerAPIApplication : Started
SequencerAPIApplication in 7.026 seconds (JVM running for 15.964)
...
2021-06-19 21:34:44.564 INFO --- [Library Tube Id=NV1234567-LIB, Flow Cell
Id=RM123DSXX] LimsApiLookupUtil : Searching for containers with name 'NV1234567-
LIB' or 'RM123DSXX'
....
2021-06-19 21:35:27.158 INFO --- [] FileService : Successfully retrieved file
'NV1234567-LIB.csv' with LIMS ID 40-51 through the API.
```

NovaSeq 6000Dx Integration v1.2 Installation

The Illumina NovaSeq 6000Dx Integration v1.2 supports the integration between Clarity LIMS and the NovaSeq 6000Dx instrument. The integration includes the following changes:

- Compatibility with Oracle Linux. For compatibility, refer to *NovaSeq 6000Dx Integration v1.2 Release Notes* on page 89.
- Security fixes.

This document provides instructions for installing NovaSeq 6000Dx Integration v1.2 and describes the components that are installed in the default configuration.

If the user is new to the NovaSeq 6000Dx integration, then follow the instructions provided in this section. If the user has installed NovaSeq 6000Dx Integration v1.1, then note that there are no user-facing changes in the configured workflow between NovaSeq 6000Dx Integration v1.1 and NovaSeq 6000Dx Integration v1.2. It is safe to use the existing NovaSeq 6000Dx v1.1 workflow installed with IPP v2.5 and reconfigure the integration for this workflow instead. For more information, refer to *[Optional] Configure application.yml Properties* on page 87.

Integration Overview

Illumina NovaSeq 6000Dx Integration v1.2 includes the following features and functionality:

- Preconfigured NovaSeqDx v1.2 workflow that maps to lab protocols and instrument runs.
- The following preconfigured protocols:
  - Run Format (NovaSeqDx v1.2)

  - NovaSeqDx Standard (NovaSeqDx v1.2) (supports the loading of pooled libraries into a library tube and the IVD sequencing run for the S2 and S4 flow cells)
  - NovaSeqDx Xp (NovaSeqDx v1.2) (supports individual lane loading on the NovaSeq 6000Dx)
  - AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2)
- Automated generation of the v2 sample sheet. This file is automatically uploaded to the sequencing instrument through the Sequencer API and is used to set up the run.
- Automated validation of run setup information. This information is uploaded to the NovaSeq 6000Dx Operating Software (NVOS) through the Sequencer API. It is used to create the run recipe and initiate the run.
- Automated tracking of the following NovaSeq 6000Dx sequencing run information, and parsing of run statistics into Clarity LIMS:
  - Progress and metrics of sequencing run
  - Per-instrument sequencing runs (tracked as part of the Run ID field value)
  - Sequencing run parameters
  - Sequencing run data directory location
  - Real-Time Analysis v3 (RTA3) version and other run-specific information
- [Optional] Preconfigured Library Prep Validation v2.3.1 workflow used for validation purposes. This workflow contains a single-step protocol that models the library prep required to produce normalized libraries that are ready for the NovaSeqDx v1.2 workflow. For more information, refer to *NovaSeq 6000Dx Integration v1.2 Validation and Troubleshooting* on page 59.

Prerequisites

Prerequisite 1: Secret Utility Configuration

If Clarity LIMS Secret Utility is not installed or configured, use the following information to configure Secret Utility:

Installation on a hosted system with Clarity LIMS (v6.2 or later)

The Clarity LIMS installation tooling configures this installation. No additional configuration is necessary.

Installation on an on-premise Clarity LIMS instance (v6.2 or later)

The installation requires the following passwords:

- Tenant DB Password
- Clarity DB Password
- FTP password
- LDAP manager password (if LDAP is enabled)

- LabLink DB password
- Reporting DB password (if installed)
- RabbitMQ password

For more information on Secret Utility configuration, refer to the *Clarity LIMS Core Functionality* documentation.

Prerequisite 2: Illumina Preset Protocols (IPP) v2.6

The Illumina NovaSeq 6000Dx Integration Package v1.2 depends on the configuration provided in Illumina Protocols (IPP) v2.6.

The installation procedure provided in this document includes steps that install the following IPP configurations on the server used for the NovaSeq 6000Dx integration installation:

- QC Protocols Base configuration
- NovaSeqDx configuration
- Library Prep Validation configuration (optional, but recommended)

For details on IPP v2.6 installation and configuration, refer to the *Illumina Preset Protocols* documentation.

Prerequisite 3: Proxy Update (Illumina Cloud Hosted Installation Only)

The Illumina cloud hosted installation configures an additional include directive for `/etc/httpd/conf.d/clarity.conf` in the proxy configuration. Additional proxy configuration files for the Sequencer API service are expected to be placed in the `/etc/httpd/conf.d/clarity` folder.

Sequencer API RPM Installation Components

NovaSeq 6000Dx Integration v1.2 is distributed as the BaseSpaceLIMS-sequencer-api RPM package. This package must be installed on the Clarity LIMS server.

The BaseSpaceLIMS-sequencer-api RPM installs the following files and scripts:

- Sequencer API WAR file
- `application.yml` configuration file
- `configure_sequencer_api_proxy.sh` configuration script
- `configure_sequencer_api_env.sh` configuration script
- `configure_sequencer_api_application.sh` configuration script

Sequencer API WAR

The RPM installs the Sequencer API WAR file into the same Tomcat container as Clarity LIMS. The WAR file is installed to the following location:
`/opt/gls/clarity/tomcat/current/webapps/Illumina#Sequencer#v2##2.6.0.0.war`

The version number can differ.

The WAR file is deployed automatically when Clarity LIMS launches. Because the RPM installs a new WAR file, Clarity LIMS is automatically stopped during installation of the RPM. Restart Clarity LIMS before installing the NovaSeq and Library Prep Validation workflow configuration from the IPP.

Configuration File

The `application.yml` configuration file is installed to the following location:

`/opt/gls/clarity/extensions/sequencer-api/application.yml`

The file properties are configured with a script during the installation process.

For a complete list of all properties included in the `application.yml` file, refer to *NovaSeq 6000Dx Integration v1.2 Configuration and User Interaction* on page 1.

Configuration Scripts

The scripts in the following table are installed to the following location:

`/opt/gls/clarity/config`

During installation, the RPM runs these scripts as they are needed.

Script Name	User	Description
`configure_sequencer_api_proxy.sh`	root	Adds configuration to the proxy to allow communication with the Sequencer API after Tomcat has been started. Run this script in the following scenarios: • When the Sequencer API is first installed. • When reconfiguring the Clarity LIMS proxy. This process happens until a version of Clarity LIMS that includes the necessary configuration files from `/etc/httpd/clarity/*.conf` is available. Not required for Illumina cloud hosted installations.
`configure_sequencer_api_env.sh`	root	Adds Secret Management setting into the Clarity LIMS Tomcat configuration. Run this script when the Sequencer API is first installed on a Secret Util-enabled Clarity LIMS.

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Script Name	User	Description
`configure_sequencer_api_application.sh`	glsjboss	• Configures the installed `application.yml` file. • Sets the Clarity LIMS URL and the key to use when signing access tokens. Run this script when the Sequencer API is first installed. If you run the script multiple times, the script reconfigures each setting to its default. The only exception is the signing key. For this setting, you are asked if you want a reset. If the reset is done, all previously issued tokens are no longer valid.

Installation Steps

If running the sequencing service and Automation Worker on the same instance, the instance must also be running a compatible version of the Oracle Linux operating system. For compatibility, refer to *NovaSeq 6000Dx Integration v1.2 Release Notes* on page 89.

[Optional] Install QC Protocols

If you are using PhiX control, install the base configuration of the QC Protocols from IPP as follows.

1. Log in as the root user on the server being used to install the integration.
2. Use the following yum command to install the IPP RPM:

```
yum install BaseSpaceLIMS-Illumina-Preset-Protocols --enablerepo=<< repo name info from support >>
```

   The `--enablerepo` command-line argument must be included to enable the repo. The Illumina Support team can provide you with the repo file with the appropriate naming convention.
3. Enter `y` to confirm that you want to proceed with the RPM and package installation.
4. Make sure that NGS Extension Package v5.23 is installed.
   If this package is not installed, you must install it or upgrade the existing package.
5. As the glsjboss user, install the `QC_Protocols.qc-protocols` configuration from the following location:

```
/opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o install QC_
Protocols.qc-protocols.
```

For information on IPP installer commands, parameters, and options, refer to the *Illumina Preset Protocols* documentation.

Install Sequencer API

Install the Sequencer API as follows.

1. On the Clarity LIMS server, log in as the root user.
2. Use the following yum command to install the RPM:

```
yum install BaseSpaceLIMS-sequencer-api --enablerepo=< repo name info from
support >
```

   The `--enablerepo` command-line argument must be included to enable the repository. The Illumina Support team can provide you with the repo file with the appropriate naming convention.

3. Enter `y` to confirm that you want to proceed with the RPM installation.
4. If necessary, install the Secret Utility Package.
   For more information on this package, refer to *Prerequisite 1: Secret Utility Configuration* on page 80.
5. As the glsjboss user, run `configure_sequencer_api_application.sh` from the following location:

```
bash /opt/gls/clarity/config/configure_sequencer_api_application.sh
```

6. Enter values for the presented as follows.
   You can also press Enter to accept the default values in the `application.yml` configuration file. For more information about the `application.yml` properties, refer to *NovaSeq 6000Dx Integration v1.2 Configuration and User Interaction* on page 1.
   - Would you like to use the apiuser user for communication with Clarity? [Y]
   - Enter required value for Token Expiry (hours) [88]
7. Update the Clarity LIMS proxy for the Sequencer API as follows.
   - For an on-premise system, run the following command:

```
bash /opt/gls/clarity/config/configure_sequencer_api_proxy.sh
```

   - For an Illumina cloud hosted system, make sure that the proxy has been updated as follows. Place the `sequencer-api.include` file in `/etc/httpd/conf.d/clarity`.

```
<Location "/Illumina/Sequencer/v2">
ProxyPass ajp://localhost:9009/Illumina/Sequencer/v2
ProxyPassReverse ajp://localhost:9009/Illumina/Sequencer/v2
</Location>
```

8. Update the Clarity LIMS Tomcat configuration as follows.

a. Make sure that Secret Utility is installed and configured. For more information, refer to *Prerequisite 1: Secret Utility Configuration* on page 80.

b. Run the following command to add the Secret Utility configuration to Clarity LIMS:

```
bash /opt/gls/clarity/config/configure_sequencer_api_env.sh
```

> This script modifies `/opt/gls/clarity/tomcat/current/bin/setenv.sh` for Clarity LIMS Tomcat to load the Secret Utility environment variable file (`/etc/profile.d/claritysecret.sh`) during startup.

c. Use the following command to Start Clarity LIMS:

```
/opt/gls/clarity/bin/run_clarity.sh start
```

Install NovaSeqDx and Library Prep Validation Workflow Configuration

Install the NovaSeqDx and Library Prep workflow configuration from IPP as follows.

1. As the glsjboss user, run the following command to install the NovaSeq 6000Dx v1.2 workflow configuration:

```
bash /opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o install
Illumina_Instruments.novaseqdx-v1.2
```

> The installer validates the import of the workflow and provides the Warning/Error details in STDOUT, which allows you to either proceed with the import or cancel it.

2. [Optional] Run the following command to install the Library Prep Validation v2.3.1 workflow:

```
/opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o install
Illumina_Instruments.Library-Prep-Validation-v2.3
```

This step is optional, but recommended. The Library Prep Validation v2.3.1 workflow allows you to validate the NovaSeqDx v1.2 workflow.

Check Sequencer API

Make sure that the Sequencer API is working as follows.

1. Visit `https://<< Clarity LIMS URL >>/Illumina/Sequencer/v2/sequencing-run/login`. This page returns the following URL string:

```
https://<< Clarity LIMS URL >>/Illumina/Sequencer/v2/oauth/authorize?client_
id=illumina_sequencer&amp;redirect_uri=../sequencer_login&amp;response_
type=token
```

2. Copy and paste the URL string into the browser. The Clarity LIMS Sign In page opens.
3. Enter sign in credentials.
4. Select Sign In. A Successfully Authenticated message displays.

[Optional] Configure `application.yml` Properties

The installer script configures the `application.yml` configuration properties by prompting for values. You can also configure these properties by editing the `application.yml` file, and then restarting Clarity LIMS. Configuring these properties is optional.

For more information, refer to *NovaSeq 6000Dx Integration v1.2 Configuration and User Interaction*.

Workflows, Protocols, and Steps Installed

NovaSeq 6000Dx Integration v1.2 includes the workflows, protocols, and steps listed in the following table.

Package	Workflow	Protocols	Steps
IPP v2.6	NovaSeqDx v1.2	Run Format (NovaSeqDx v1.2)	1. Define Run Format (NovaSeqDx v1.2)
		NovaSeqDx Standard (NovaSeqDx v1.2)	1. Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.2) 2. Dilute and Denature (NovaSeqDx v1.2)
		NovaSeqDx Xp (NovaSeqDx v1.2)	1. Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.2) 2. Dilute, Denature & ExAmp (NovaSeqDx v1.2) 3. Load to Flowcell (NovaSeqDx v1.2)
		AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2)	1. AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.2)
IPP v2.6	Library Prep Validation v2.3.1	Library Prep Validation v2.3.1	1. Library Prep Validation v2.3.1

For detailed descriptions of the steps and automations included in each protocol, and details on other components in the configuration, refer to *NovaSeq 6000Dx Integration v1.2 Configuration and User Interaction* on page 1.

For instructions on using the Library Prep Validation v2.3.1 protocol, refer to *NovaSeq 6000Dx Integration v1.2 Validation and Troubleshooting* on page 59.

Instrument Software

The NovaSeq 6000Dx instrument software includes the following components:

- NovaSeq 6000Dx Operating Software (NVOS)—Contains the user interface for setting up the sequencing run. Responsible for controlling the instrument and acquiring the images.
- Real-Time Analysis v3 (RTA3)—Takes the images generated by the first module, processes, and analyzes them. Makes sure that data files are created and copied to the final destination folder.
- Sequencing Analysis Viewer (SAV)—Displays the important quality metrics generated by the RTA3 software.
- NovaSeq 6000Dx recipes—Provides system operation instructions for use with NovaSeq 6000Dx reagent kits for SP, S1, S2, and S4 flow cells.
- Universal Copy Service—Copies output files to destinations such as final destination folder and/or BaseSpace Sequence Hub (when the instrument is configured for use with BaseSpace Sequence Hub).

Instrument Integration Configuration

For the NovaSeq 6000Dx API integration to work, the instrument must be able to communicate with Clarity LIMS through the API. Complete the following steps to configure the NVOS and confirm that you can access Clarity LIMS from the instrument.

1. Launch NVOS and wait for the initialization process to complete.
2. On the NVOS Instrument Settings page, do as follows.
   a. Select the Global Settings tab.
   b. Under Instrument Mode, select one of the following modes:
      - Allow users to switch between IVD and RUO
      - IVD Only
      - RUO Only
3. If you selected IVD Only instrument mode, select the IVD Settings tab and complete the following steps:
   a. Configure Run Setup as follows.
      - Select the Select runs from the LIMS server option.
      - Enter the Clarity LIMS URL (eg, `https://yourserver.claritylims.com`).
   b. [Optional] Under Output Location, enter the output folder location. You can also specify the output folder location when setting up a run in NVOS.

4. If you selected RUO Only instrument mode, select the RUO Settings tab and complete the following steps:
   a. Configure Run Setup as follows.
      - Select the Select runs from the LIMS server option.
      - Enter the Clarity LIMS URL(eg, `https://yourserver.claritylims.com`).
   b. Configure Default Work Type as follows.
      - [Optional] If selection support between NovaSeq Xp or NovaSeq Standard is required for the workflow, select the Enable workflow selection checkbox.
      - Select the required default workflow type: NovaSeq Standard or NovaSeq Xp.
   c. [Optional] Configure Output Location as follows.
      - [Optional] Enter the output folder location. You can also specify the output folder location when setting up a run in NVOS.
      - [Optional] To upload and store run data in the cloud server, select the BaseSpace Sequence Hub checkbox. Select a Configuration option (Run Monitoring and Storage or Run Monitoring Only) and select your Hosting Location from the drop-down list.
5. Select Save to complete the configuration.
6. On the NVOS home page, you can now select Sequence to log into Clarity LIMS and start a run.

NovaSeq 6000Dx Integration v1.2 Release Notes

These Release Notes describe the key changes to software components for the Clarity LIMS NovaSeqDx Integration Package v1.2.

Compatibility

This version of the integration package is compatible with the following software:

- BaseSpace Clarity LIMS v6.2 and later
- NGS Extensions Package v5.23 and later
- Illumina Preset Protocols (IPP) v2.6.0 and later
- NovaSeq 6000Dx Operating Software (NVOS) v2.3.1
- Oracle Linux (64-bit) v8.8

New Features

Updated Groovy to v3.0.7.

Defects Fixed

During configuration, you can now validate the existence of the input user instead of resetting the user and password.

Known Issues

None

NovaSeq 6000Dx Integration v1.1 Configuration and User Interaction

Illumina NovaSeqDx Integration Package v1.1 supports the integration of Clarity LIMS to Illumina NovaSeq 6000Dx instruments. This integration supports Clarity LIMS v5.1 and later.

For instructions on validating and troubleshooting Illumina NovaSeqDx Integration Package v1.1, refer to *NovaSeq 6000Dx Integration v1.1 Validation and Troubleshooting* on page 148.

The configuration provided in this integration has been established to support NovaSeq 6000Dx lab processes. Any configuration changes to protocols or workflows—including renaming protocols, steps, and fields—could break the process.

Overview

The integration includes the following features:

- Preconfigured NovaSeqDx v1.1 workflow that maps to lab protocols and instrument runs.
- The following preconfigured protocols:
  - Run Format (NovaSeqDx v1.1)
  - NovaSeqDx Standard (NovaSeqDx v1.1) (supports the loading of pooled libraries into a library tube)
  - NovaSeqDx Xp (NovaSeqDx v1.1) (supports individual lane loading on the NovaSeq)
  - AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1)
- Automated generation of v2 sample sheet. This file is automatically uploaded to the instrument via the API.
- Automatic validation of run setup information. This information is used to create the run recipe and initiate the run.
- Automated tracking of the NovaSeqDx sequencing run and parsing of run statistics into Clarity LIMS, including the following features:
  - Progress and metrics of sequencing run
  - Per-instrument sequencing runs (tracked as part of the Run ID field value)
  - Sequencing run parameters
  - Sequencing run data directory location
  - Real-Time Analysis v3 (RTA3) version and other run-specific information
  - [Optional] Preconfigured Library Prep Validation v2.3.0 workflow used for validation purposes only. The workflow contains a single-step protocol that models the library prep required to produce normalized libraries that are ready for the NovaSeqDx v1.1 workflow. For more

information, refer to *NovaSeq 6000Dx Integration v1.1 Validation and Troubleshooting* on page 148. The Library Prep Validation v2.3.0 workflow is available in the Illumina Preset Protocols (IPP) v2.5 package.

Prerequisites and Assumptions

Samples enter the NovaSeqDx v1.1 workflow as normalized libraries. It is assumed that the following steps have completed before samples are assigned to the workflow:

- Samples have been accessioned into the Clarity LIMS.
- Samples have been run through QC and library prep.
- Samples have been normalized, and the value is captured in a field called Normalized Molarity (nM).

For more information on sample accessioning, refer to *Sample Accessioning* and *Upload and Modify Samples* in the Getting Started section of the Clarity LIMS Core Functionality documentation.

You can assign samples to workflows automatically, using a routing script, or manually—from the Projects & Samples dashboard. Refer to *Assign and Process Samples* in the Clarity LIMS Core Functionality documentation.

Workflows, Protocols, and Steps

The Illumina NovaSeq 6000Dx Integration Package v1.1 includes the following workflows:

- NovaSeqDx v1.1
- Library Prep Validation v2.3.0 (optional, but recommended for validation purposes)

The following table describes the protocols and steps included in these workflows.

Table 60 NovaSeq 6000Dx Integration Package v1.1 Protocols and Steps

Protocol Name	Protocol Purpose	Protocol Steps
Library Prep Validation v2.3.0 Workflow		
Library Prep Validation v2.3.0	Included for validation purposes only, this protocol models the library prep steps required to advance samples to the Run Format (NovaSeqDx v1.1) protocol. The protocol contains a single step - Library Prep Validation v2.3.0. After this step, a routing script sends the samples to the first step of the NovaSeqDx v1.1 workflow - Define Run Format (NovaSeqDx v1.1).	1. Library Prep Validation v2.3.0
NovaSeqDx v1.1 Workflow		

Protocol Name	Protocol Purpose	Protocol Steps
Run Format (NovaSeqDx v1.1)	Allows for the assignment of per sample values for the following properties: • NovaSeqDx Run Mode: Select either RUO or DX. • Loading Workflow Type: Select either NovaSeq Standard or NovaSeq Xp. • Normalized Molarity (nM): Enter a value for each sample. • Flowcell Type: Select from options SP, S1, S2, or S4. • Final Loading Concentration (pM): Select from options 225 (PCR-free workflows) or 400 (Nano workflows), or enter a different value. Compares the Normalized Molarity value of each sample with the Minimum Molarity value. Routing script sends samples to the NovaSeqDx Standard or NovaSeqDx Xp protocol, according to the selected Loading Workflow Type. Samples with Normalized Molarity less than Minimum Molarity are removed from the workflow.	1. Define Run Format (NovaSeqDx v1.1)
NovaSeqDx Standard (NovaSeqDx v1.1)	Samples are pooled and added to the library tube in preparation for the NovaSeq 6000Dx run. The run setup information is validated and a sample sheet is generated. Routing script sends the library tube to the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1) protocol.	1. Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.1) 2. Dilute and Denature (NovaSeqDx v1.1)

Protocol Name	Protocol Purpose	Protocol Steps
NovaSeqDx Xp (NovaSeqDx v1.1)	Samples are pooled and added to lanes on the NovaSeq 6000Dx flow cell. The option selected in the Define Run Format (NovaSeqDx v1.1) step determines the flow cell type. The run setup information is validated and a sample sheet is generated. Routing script sends flow cell to the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1) protocol.	1. Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.1) 2. Dilute, Denature & ExAmp (NovaSeqDx v1.1) 3. Load to Flowcell (NovaSeqDx v1.1)
AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1)	All samples complete the workflow by going through this protocol. This protocol contains one fully automated step. Do not add samples to the Ice Bucket or start the step. The integration starts the step automatically.	1. AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1)

Validation Workflow

The Library Prep Validation v2.3.0 workflow allows for validation of the system after installation is complete. For details, refer to *NovaSeq 6000Dx Integration v1.1 Validation and Troubleshooting* on page 148.

Step Automations

The following table lists the step automations included in the NovaSeqDx v1.1 workflow. Automation details are provided in the documentation for each step.

For details on the validation workflow, refer to *NovaSeq 6000Dx Integration v1.1 Validation and Troubleshooting* on page 148.

Table 61 NovaSeq 6000Dx v1.1 Workflow Step Automations

Protocol	Step	Automation
1. Run Format (NovaSeqDx v1.1)	1. Define Run Format (NovaSeqDx v1.1)	Routing Script Set Next Steps

Protocol	Step	Automation
2. NovaSeqDx Standard (NovaSeqDx v1.1)	1. Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.1)	Calculate Volumes Set Next Step Validate Inputs Flowcell Type and Single Pool Validate Single NovaSeqDx Run Mode Validate Unique Indexes (Not used. The core Clarity LIMS configuration for pooling handles this functionality.)
	2. Dilute and Denature (NovaSeqDx v1.1)	Routing Script Validate Library Tube Barcode Validate Run Setup and Generate Sample Sheet Validate Single Input
3. NovaSeqDx Xp (NovaSeqDx v1.1)	1. Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.1)	Calculate Volumes Set Next Step Validate Inputs Flowcell Type and Single Pool Validate Unique Indexes (Not used. The core Clarity LIMS configuration for pooling handles this functionality.)
	2. Dilute, Denature, and ExAmp (NovaSeqDx v1.1)	Calculate Volumes Set Next Step Validate Inputs Flowcell Type
	3. Load to Flowcell (NovaSeqDx v1.1)	Validate Flowcell Barcode Validate Inputs and Selected Container Validate Run Setup and Generate Sample Sheet
4. AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1)	1. AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1)	Update Lane Number Set Next Steps (Not used. By default, the Sequencer API determines this functionality. If necessary, enable the automation and use it to override the next step behavior used by the Sequencer API.)

Terminology

As of Clarity LIMS v5, the term user-defined field (UDF) has been replaced with custom field in the user interface. However, the API resource is still called UDF.

There are two types of custom fields:

- Master step fields—Configured on master steps. Master step fields only apply to the following:
  - The master step on which the fields are configured.
  - The steps derived from those master steps.
- Global fields—Configured on entities (eg, submitted sample, derived sample, measurement, etc.). Global fields apply to the entire Clarity LIMS system.

Protocol 1: Run Format (NovaSeqDx v1.1)

This protocol sets the Loading Workflow Type and allows the choice of the appropriate Flowcell Type and Final Loading Concentration (pM). After the protocol, a routing script sends the normalized libraries to either the NovaSeqDx Standard (NovaSeqDx v1.1) or the NovaSeqDx Xp (NovaSeqDx v1.1) protocol.

This protocol contains one step: Define Run Format (NovaSeqDx v1.1).

Step 1. Define Run Format (NovaSeqDx v1.1)

Step input: Normalized Template Plate (NTP, normalized libraries)

Step output: None

Set Next Steps Automation

Automatically triggered on exit of the Record Details screen, this automation completes the following actions:

- Sets the next step for samples to REMOVE:

```
nextStep = ::REMOVE::
```

- Checks NovaSeqDx Run Mode, Loading Workflow Type, and Flowcell Type. Only the NovaSeq Standard with the S2/S4 flow cell is supported for the NovaSeqDx Run Mode:

```
if (input.::NovaSeqDx Run Mode:: == ::DX::) {
    if (input.::Loading Workflow Type:: == ::NovaSeq Xp::) {
        fail(::Invalid option selected. NovaSeq Standard shall be selected for DX
        mode.::);
    };
    if (input.::Flowcell Type:: == ::SP:: || input.::Flowcell Type:: == ::S1::) {
        fail(::Invalid option selected. Only S2 or S4 flowcell shall be selected
        for DX mode.::);
```

```
    };
};
```

- Calculates the Minimum Molarity with the following formula:

```
input.::Minimum Molarity (nM):: = (5 * input.::Final Loading Concentration
(pM)::)/1000
```

- Checks Normalized Molarity value. For samples with no Normalized Molarity value (eg, empty value, not including 0), generates an error message informing that the field cannot be empty:

```
if (!input.hasValue(::Normalized Molarity (nM)::)) {
  fail(::The Normalized Molarity cannot be empty.::) ;
}
```

- Compares the Normalized Molarity value of each sample with the Minimum Molarity value. If the Normalized Molarity value is lower than the Minimum Molarity value, this sets the Loading Workflow Type of the sample to [Remove from workflow]. The automation also records a message in the Warning field for the sample:

```
else if (input.::Normalized Molarity (nM):: < input.::Minimum Molarity (nM)::) {
    input.::Warning:: = ::The Normalized Molarity is too low.::;
    input.::Loading Workflow Type:: = ::[Remove from workflow]::;
} else {
    input.::Warning:: = ::n/a::
}
```

At this point, the following options are available:

- Correct the NovaSeqDx Run Mode and Normalized Molarity value on the Record Details screen. Edit the Loading Workflow Type field and set it to NovaSeq Standard or NovaSeq Xp, as applicable.
- Complete the protocol without correcting the Normalized Molarity value. In this case, those samples are removed from the workflow.

Routing Script Automation

Automatically triggered on exit of the step, this automation invokes the changeWorkflow script, which routes step inputs appropriately.

- Samples with Loading Workflow Type field value = NovaSeq Standard are routed to the NovaSeqDx v1.1 workflow. Then, the samples are queued for the Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.1) step.
- Samples with Loading Workflow Type field value = NovaSeq Xp are routed to the NovaSeqDx v1.1 workflow. Then, the samples are queued for the Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.1) step.

Default automation command line is as follows.

```
bash -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar
-u {username} -p {password} -i {stepURI:v2} -l {compoundOutputFileLuid0}
script:changeWorkflow \
\
--FIELD_NAME 'Loading Workflow Type' \
--FIELD_VALUE 'NovaSeq Standard' \
--WORKFLOW 'NovaSeqDx v1.1' \
--STEP 'Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.1)' \
--INPUTS_OR_OUTPUTS 'INPUTS' \
\
--FIELD_NAME 'Loading Workflow Type' \
--FIELD_VALUE 'NovaSeq Xp' \--WORKFLOW 'NovaSeqDx v1.1' \
--STEP 'Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.1)' \
--INPUTS_OR_OUTPUTS 'INPUTS'"
```

Master Step Fields

There are four fields defined on the Define Run Format (NovaSeqDx v1.1) master step.

The following table lists field configuration details.

Table 62 Define Run Format (NovaSeqDx v1.1) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Comment	Multiline Text		None
Flowcell Type	Text Dropdown	Required Field Custom Entries	Presets • SP • S1 • S2 • S4
Instruction	Text	Read Only	Default • Add Flowcell Type, Loading Workflow Type, and NovaSeqDx Run Mode
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp

For Research Use Only. Not for use in diagnostic procedures.

Global Fields

The following table lists the global custom fields that are configured to display on the Define Run Format (NovaSeqDx v1.1) step.

Table 63 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
Adjusted Per Sample Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Final Loading Concentration (pM)	Numeric Dropdown	Required Field Custom Entries	Presets • 225 • 400 Decimal places displayed = 0
Flowcell Type	Text Dropdown	Required Field	Presets • SP • S1 • S2 • S4
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
Minimum Molarity (nM)	Numeric		Decimal places displayed = 2
Normalized Molarity (nM)	Numeric		Decimal places displayed = 2
NovaSeqDx Run Mode	Text Dropdown	Required Field	Presets • DX • RUO
Per Sample Volume (µl)	Numeric	Read Only	Decimal places displayed = 2

Field Name	Field Type	Options	Additional Options and Dropdown Items
Warning	Text Dropdown	Read Only Custom Entries	Presets • The Normalized Molarity (nM) is too low. • Not applicable

User Interaction

Refer to Run Define Run Format (NovaSeqDx v1.1) step section in *NovaSeq 6000Dx Integration v1.1 Validation and Troubleshooting* on page 148.

Protocol 2: NovaSeqDx Standard (NovaSeqDx v1.1)

Samples are routed to this protocol when their Loading Workflow Type value is set to NovaSeq Standard. Samples are pooled and added to a library tube in preparation for the NovaSeqDx Run.

At the end of this protocol, a routing script sends the library tube to the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1) protocol.

This protocol contains the following two steps:

- Step 1: Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.1)
- Step 2: Dilute and Denature (NovaSeqDx v1.1)

Step 1: Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.1)

In this step, libraries are placed manually into a single pool. Resuspension buffer and reagents are added.

Create only one pool per step.

Step input: NTP (normalized libraries)

Step output: Bulk pool

Validate Unique Indexes automation is not used. The core Clarity LIMS configuration for pooling handles this functionality.

Validate Single NovaSeqDx Run Mode

The Validate Single NovaSeqDx Run Mode automation is triggered on entry of the step. The following automation checks that all input samples have the same NovaSeqDx Run Mode assigned:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar \
```

```
script:validate_same_analyte_udf_value_for_all_inputs \
-i {stepURI:v2} \
-u {username} \
-p {password} \
-l {compoundOutputFileLuid1} \
-f 'NovaSeqDx Run Mode'"
```

Validate Inputs Flowcell Type and Single Pool Automation

Automatically triggered on exit of the Pooling screen, this automation completes the following actions:

- Checks that all samples in the pool have the same Flowcell Type assigned to them.
- Checks that only one pool has been created.
- Changes the value of error and logging messages to reference the type of pool passed as bulk pool.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar \
script:validate_flowcell_for_input_pools \
-i {stepURI:v2} \
-u {username} \
-p {password} \
-l {compoundOutputFileLuid1} \
-validateSingleOutput true \
-poolType bulk"
```

Calculate Volumes Automation

Automatically triggered when you select the Calculate Volumes button on the Record Details screen, this automation completes the following actions:

- Calculates the number of samples in the pool.

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: + 1
```

- Sets the value of the Bulk Pool Volume (µl), and PhiX Volume (µl) field based on the selected Flowcell Type.

```
step.::PhiX Volume (ul):: = !step.hasValue(::% PhiX (2.5nM) Spike-In::) ? 0 :
step.::% PhiX (2.5nM) Spike-In::;
if (input.::Flowcell Type:: == ::SP::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 100;
step.::PhiX Volume (ul):: *= 0.6
};
if (input.::Flowcell Type:: == ::S1::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 100;
```

```
step.::PhiX Volume (ul):: *= 0.6
};
if (input.::Flowcell Type:: == ::S2::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 150;
step.::PhiX Volume (ul):: *= 0.9
};
if (input.::Flowcell Type:: == ::S4::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 310;
step.::PhiX Volume (ul):: *= 1.9
};
if (step.::PhiX Volume (ul):: == 0) {
step.::PhiX Volume (ul):: = ::::
};
```

- Calculates the Per Sample Volume (µl) to be added to the pool.

```
input.::Per Sample Volume (ul):: = ( ( (input.::Final Loading Concentration
(pM):: * 5 / 1000) / input.::Normalized Molarity (nM):: ) * step.::Bulk Pool
Volume (ul):: ) / step.::Number of Samples in Pool::
```

> For accurate pipetting of each sample in a pool for sequencing, the Per Sample Volume (µl) value must be equal to or higher than the Minimum Per Sample Volume (µl). The default value (set at 5) can be edited.

Assuming the default Minimum Per Sample Volume (µl) value of 5, this automation completes the following steps for a given batch:

1. If the smallest Per Sample Volume (µl) value is less than 5, automatically assigns a value of 5 to the Adjusted Per Sample Volume (µl) field of the sample.
2. Adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

- Calculates the Total Sample Volume (µl) field value.

```
step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: +
input.::Adjusted Per Sample Volume (ul)::
```

- If the Total Sample Volume is less than the Bulk Pool Volume, this automation calculates the RSB Volume (µl) field value.

```
if (step.::Total Sample Volume (ul):: >= step.::Bulk Pool Volume (ul)::) {
output.::RSB Volume (ul):: = 0
}
else {
output.::RSB Volume (ul):: = step.::Bulk Pool Volume (ul):: - step.::Total
Sample Volume (ul)::
} ;
```


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- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs.

```
output.::Flowcell Type:: = input.::Flowcell Type:: ;
output.::Loading Workflow Type:: = input.::Loading Workflow Type:: ;
```

- Sets the Volume of Pool to Denature (µl) value a calculates the NaOH Volume (µl) and Tris-HCl Volume (µl) values based on the Flowcell Type.

```
if ( input.::Flowcell Type:: == ::SP:: ) {
output.::Volume of Pool to Denature (ul):: = 100 ;
output.::NaOH Volume (ul):: = 25 ;
output.::Tris-HCl Volume (ul):: = 25
} ;
if ( input.::Flowcell Type:: == ::S1:: ) {
output.::Volume of Pool to Denature (ul):: = 100 ;
output.::NaOH Volume (ul):: = 25 ;
output.::Tris-HCl Volume (ul):: = 25
} ;
if ( input.::Flowcell Type:: == ::S2:: ) {
output.::Volume of Pool to Denature (ul):: = 150 ;
output.::NaOH Volume (ul):: = 37 ;
output.::Tris-HCl Volume (ul):: = 38
} ;
if ( input.::Flowcell Type:: == ::S4:: ) {
output.::Volume of Pool to Denature (ul):: = 310 ;
output.::NaOH Volume (ul):: = 77;
output.::Tris-HCl Volume (ul):: = 78
}
```

- Uses the `NovaSeq_Standard_Bulk_Pool1.csv`, `NovaSeq_Standard_Bulk_Pool2.csv`, and `NovaSeq_Standard_Bulk_Pool3.csv` template files to generate a single CSV file containing information about the pool and the samples it contains. The generated file is available for download in the Files section of the Record Details milestone in the Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.1) step.

```
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} \
   script:driver_file_generator \
      -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Standard_
      Bulk_Pool1.csv \
      -o 1.csv \
   script:driver_file_generator \
```

```
        -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Standard_
        Bulk_Pool2.csv \
        -o 2.csv \
    script:driver_file_generator \
        -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Standard_
        Bulk_Pool3.csv \
        -o 3.csv \
&& cat 1.csv 2.csv 3.csv > {compoundOutputFileLuid0}.csv
```

- Resets the Total Sample Volume (µl) and Number of Samples in Pool field values so that the automation is idempotent.

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: - 1;
step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: -
input.::Adjusted Per Sample Volume (ul)::
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, the following automation sets the next step for samples to ADVANCE, advancing them to the Dilute and Denature (NovaSeqDx v1.1) step in the protocol:

```
nextStep = ::ADVANCE::
```

The following automation copies the NovaSeqDx Run Mode values from the step inputs to the step outputs:

```
output.::NovaSeqDx Run Mode:: = input.::NovaSeqDx Run Mode::
```

Master Step Fields

There are seven fields defined on the Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.1) step. The following table lists configuration details.

Table 64 Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.1) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
% PhiX (2.5 nM) Spike-In	Numeric		Range = 0–100 Decimal places displayed = 0

Field Name	Field Type	Options	Additional Options and Dropdown Items
Bulk Pool Volume (µl) (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 2
Number of Flowcells to Sequence	Numeric	Required Field	Range = 1–10 Decimal places displayed = 0
Minimum Per Sample Volume (µl)	Numeric	Required Field	Decimal places displayed = 2 Default • 5
Number of Samples in Pool (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 0 Default • 0
PhiX Volume (µl) (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 2
Total Sample Volume (µl) (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 2 Default • 0

Global Fields

The following table lists the global custom fields that are configured to display on the Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.1) step.

Table 65 Global Custom Fields Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			

Field Name	Field Type	Options	Additional Options and Dropdown Items
Flowcell Type	Text Dropdown	Required Field	Presets • SP • S1 • S2 • S4
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
NaOH Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
RSB Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Tris-HCl Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Volume of Pool to Denature (µl) (Used in Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.1) step only. Displays on Record Details screen and in the generated CSV file.)	Numeric	Read Only	Decimal places displayed = 0

User Interaction

Refer to Run Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.1) step section in *NovaSeq 6000Dx Integration v1.1 Validation and Troubleshooting* on page 148.

Step 2: Dilute and Denature (NovaSeqDx v1.1)

In this step, the addition of NaOH, Tris-HCl, and Resuspension Buffer (RSB) denatures and dilutes pooled samples. Manually place the pooled samples into the library tube for the NovaSeqDx Run.

In addition, this step validates the run setup information and generates the sample sheet file.

Step input: Bulk pool

Step output: Library tube

Validate Single Input Automation

Automatically triggered at the beginning of the step, this automation checks that there is only one container input to the step.

```
script:validateSampleCount -min 1 -max 1
```

Validate Library Tube Barcode Automation

Automatically triggered on exit of the Placement screen, this automation completes the following actions:

- Validates the library tube barcode to ensure it conforms to the following barcode masks:
  - DX mode: DX[0-9]{7}-[A-Z]{3}
  - RUO mode: [A-Z]{2}[0-9]{7}-[A-Z]{3}

```
if ((input.::NovaSeqDx Run Mode:: == ::DX:: && !output.container.name.matches
(::^DX[0-9]{7}-[A-Z]{3}$::)) || (input.::NovaSeqDx Run Mode:: == ::RUO:: &&
!output.container.name.matches(::[A-Z]{2}[0-9]{7}-[A-Z]{3}::))) {
fail(::Invalid Library Tube Barcode. Please verify and try again.::)
}
```

- Copies Flowcell Type and Loading Workflow Type field values from step inputs to outputs:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ;
output.::Loading Workflow Type:: = input.::Loading Workflow Type::
```

Validate Run Setup and Generate Sample Sheet Automation

Triggered by a button on the Record Details screen, this automation completes the following actions:

> The script definition portion of Validate Run Setup has been moved into the Validation Script custom field due to the 4000 character limit. If modification is required on Validate Run Setup script, perform the changes directly on the Validation Script custom field default value.

- Copies the Flowcell Type from the step input to the Run Mode field (hidden):

```
step.::Run Mode:: = input.::Flowcell Type::
```

- Validates the parameters entered on the Record Details screen, which is used to set up the run and generate the sample sheet file. The parameters are as follows. For more information, refer to the *Respond to Validation / Recipe Request Call from Instrument* section of *NovaSeq 6000Dx Run* on page 135.
  - Experiment Name only contains alphanumeric, dash, or underscore characters. Spaces are not permitted.
  - When Workflow Type = No Index, both Index Read 1 and Index Read 2 must be zero.
  - When Workflow Type = Single Index, Index Read 1 must be greater than zero, and Index Read 2 must be zero.

  - When Workflow Type = Dual Index, both Index Read 1 and Index Read 2 must be greater than zero.

```
if(!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) {
fail(::Experiment Name contains prohibited characters. Allowed characters are:
a-z, A-Z, 0-9, -, and _::)
};
if (step.::Workflow Type:: == ::No Index::) {
if (step.::Index Read 1:: != 0 || step.::Index Read 2:: != 0) {
fail(::Index Read 1 and Index Read 2 must be 0 if No Index is selected.::)
}
} else if (step.::Workflow Type:: == ::Single Index::) {
if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) {
fail(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if Single
Index is selected.::)
}
} else {
if (step.::Index Read 1:: == 0 || step.::Index Read 2:: == 0) {
fail(::Index Read 1 and Index Read 2 must be greater than 0 if Dual Index is
selected.::)
}
};
```

- Checks the Paired End and Read 2 Cycles field values as follows.
  - If Paired End = True, both Read 1 Cycles and Read 2 Cycles must be greater than 0.
  - If Paired End = False, Read 2 Cycles must be zero.

```
if (step.::Paired End::.toBoolean()) {
   if (step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) {
      fail(::Read 1 Cycles and Read 2 Cycles must be greater than 0 if Paired
      End is True.::)
   }
} else {
   if (step.::Read 2 Cycles:: != 0) {
      fail(::Read 2 Cycles must be 0 if Paired End is False.::)
   }
};
```

- Checks the Flowcell field value as follows.
  - If Flowcell Type value is not SP, checks that the values of Read 1 Cycles and Read 2 Cycles are each 151 or less. If the value is greater than 151, an error message is generated.

```
if (input.::Flowcell Type::!=::SP:: && step.::Read 1 Cycles:: > 151) {
fail(::Read 1 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
```


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```
};
if (input.::Flowcell Type::!=::SP:: && step.::Read 2 Cycles:: > 151) {
fail(::Read 2 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
};
```

- Validate Analysis Software Version to ensure it is a valid version string.

```
if (step.hasValue(::Analysis Software Version::) && !step.::Analysis Software
Version::.matches(::^(\\d+)(\\.\\d+)*$::)) {
fail(::Analysis Software Version contains prohibited characters. Allowed
characters are 0-9 and period. It shall start and end with numbers and separated
by single period e.g. 3.8.4::)
};
```

- Validate Override Cycles value to contain only Y, N, I, U, 0-9, and semicolon characters.

```
if (step.hasValue(::Override Cycles::) && !step.::Override Cycles::.matches(::
[YNIU0-9;]+::)) {
fail(::Override Cycles contains prohibited characters. Allowed characters are:
Y, N, I, U, 0-9 and ;. Example: N1Y150;I8;I7N1;Y141U10.::)
};
```

- Sets the next step for samples to REMOVE:

```
nextStep = ::REMOVE::
```

- Generates the V2 sample sheet and attaches it to the step. For more information, refer to *Sample Sheet Generation* on page 134.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeqDx_BCL2FASTQ_
Samplesheet_v2.csv \
-o {compoundOutputFileLuid0}.csv \
-q true \
-destLIMSID {compoundOutputFileLuid0} \
-l {compoundOutputFileLuid1}"
```

Routing Script Automation

Automatically triggered on exit of the step, this automation invokes the changeWorkflow script, which routes step outputs to the NovaSeqDx v1.1 workflow, and queues them for the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1) step.

The default automation command line is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar
-u {username} -p {password} -i {stepURI:v2} -l {compoundOutputFileLuid1}
script:changeWorkflow \
--FIELD_NAME 'N/A' \
--FIELD_VALUE 'N/A' \
--WORKFLOW 'NovaSeqDx v1.1' \
--STEP 'AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1)' \
--INPUTS_OR_OUTPUTS 'OUTPUTS'"
```

Master Step Fields

There are 19 fields defined on the Dilute and Denature (NovaSeqDx v1.1) step. These fields are required for sample sheet and JSON file generation.

The following table lists field configuration details.

Table 66 Dilute and Denature (NovaSeqDx v1.1) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Analysis Software Version	Text		
BaseSpace Sequence Hub Configuration	Text Dropdown	Required Field	Not Used Run Monitoring Only Run Monitoring and Storage
Custom Recipe Path	Text		
Experiment Name	Text	Required Field	
Index Read 1	Numeric Dropdown	Required Field Custom Entries	Range = 0–20 Decimal places displayed = 0 Presets • 0 • 6 • 8

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Field Name	Field Type	Options	Additional Options and Dropdown Items
Index Read 2	Numeric Dropdown	Required Field Custom Entries	Range = 0–20 Decimal places displayed = 0 Presets • 0 • 6 • 8
Output Folder	Text	Required Field	
Override Cycles	Text		
Paired End	Text Dropdown	Required Field	Presets • True • False
Read 1 Cycle	Numeric Dropdown	Required Field Custom Entries	Range = 1–251 Decimal places displayed = 0 Presets • 251* • 151 • 101 • 51 *Value of 251 is only supported for SP flow cell type. For all other cell types, maximum value is 151.
Read 2 Cycle	Numeric Dropdown	Required Field Custom Entries	Range = 0–251 Decimal places displayed = 0 Presets • 251* • 151 • 101 • 51 *Value of 251 is only supported for SP flow cell type. For all other cell types, maximum value is 151.

Field Name	Field Type	Options	Additional Options and Dropdown Items
Run Mode (Not displayed in user interface)	Text Dropdown	Read Only	Presets • SP • S1 • S2 • S4
Use Custom Index Read 1 Primer	Toggle Switch		Default • None Set
Use Custom Read 1 Primer	Toggle Switch		Default • None Set
Use Custom Read 2 Primer	Toggle Switch		Default • None Set
Use Custom Recipe	Toggle Switch	Required Field	Default • No
Validation Script	Multiline Text	Required Field Read Only	Default value is provided in the drop-down section that follows the table. Do not remove this field as it is used by Validate Run Setup and Generate Sample Sheet automation script.
Workflow	Text	Read Only	Default • GenerateFASTQ
Workflow Type	Text Dropdown	Required Field	Presets • No Index • Single Index • Dual Index • Custom

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Validation Script

```
step.::Run Mode:: = input.::Flowcell Type::; if (!step.::Experiment
Name::.matches(::[a-zA-Z0-9-_]+::)) { fail(::Experiment Name contains prohibited
characters. Allowed characters are: a-z, A-Z, 0-9, -, and _::) }; if
(step.::Workflow Type:: == ::No Index::) { if (step.::Index Read 1:: != 0 ||
step.::Index Read 2:: != 0) { fail(::Index Read 1 and Index Read 2 must be 0 if
No Index is selected.::) } } else if (step.::Workflow Type:: == ::Single
Index::) { if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) { fail
(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if Single
Index is selected.::) } } else { if (step.::Index Read 1:: == 0 || step.::Index
Read 2:: == 0) { fail(::Index Read 1 and Index Read 2 must be greater than 0 if
Dual Index is selected.::) } }; if (step.::Paired End::.toBoolean()) { if
(step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) { fail(::Read 1
Cycles and Read 2 Cycles must be greater than 0 if Paired End is True.::) } }
else { if (step.::Read 2 Cycles:: != 0) { fail(::Read 2 Cycles must be 0 if
Paired End is False.::) } }; if (input.::Flowcell Type:: != ::SP:: &&
step.::Read 1 Cycles:: > 151) { fail(::Read 1 Cycles must not be larger than 151
if it is not SPrime Flowcell::) }; if (input.::Flowcell Type:: != ::SP:: &&
step.::Read 2 Cycles:: > 151) { fail(::Read 2 Cycles must not be larger than 151
if it is not SPrime Flowcell::) }; if (step.hasValue(::Analysis Software
Version::) && !step.::Analysis Software Version::.matches(::^(\\d+)
(\\.\\d+)*$::)) { fail(::Analysis Software Version contains prohibited
characters. Allowed characters are 0-9 and period. It shall start and end with
numbers and separated by single period e.g. 3.8.4::) }; if (step.hasValue
(::Override Cycles::) && !step.::Override Cycles::.matches(::[YNIU0-9;]+::)) {
fail(::Override Cycles contains prohibited characters. Allowed characters are:
Y, N, I, U, 0-9 and ;. Example: N1Y150;I8;I7N1;Y141U10.::) }; nextStep =
::REMOVE::
```

Global Fields

The following table lists the global custom fields that are configured to display on the Dilute and Denature (NovaSeqDx v1.1) step.

Table 67 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			

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Field Name	Field Type	Options	Additional Options and Dropdown Items
Flowcell Type	Text Dropdown	Required Field	Presets • SP • S1 • S2 • S4
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User Interaction

Refer to Run Dilute and Denature (NovaSeqDx v1.1) step section in *NovaSeq 6000Dx Integration v1.1 Validation and Troubleshooting* on page 148.

Protocol 3: NovaSeqDx Xp (NovaSeqDx v1.1)

Samples are routed to this protocol when their Loading Workflow Type value is set to NovaSeq Xp.

Samples are pooled and added to lanes on the NovaSeqDx flow cell type selected in the Define Run Format (NovaSeqDx v1.1) step. At the end of this protocol, the flow cell is sent to the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1) protocol.

This protocol contains the following three steps:

- Step 1: Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.1)
- Step 2: Dilute, Denature, and ExAmp (NovaSeqDx v1.1)
- Step 3: Load to Flowcell (NovaSeqDx v1.1)

Step 1: Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.1)

Manually place libraries into a pool.

Create only one pool per step.

Step input: NTP (normalized libraries)

Step output: Bulk pool

Validate Unique Indexes automation is not used. The core Clarity LIMS configuration for pooling handles this functionality.

Validate Inputs Flowcell Type and Single Pool Automation

Automatically triggered on exit of the Pooling screen, this automation completes the following actions:

- Checks that all samples in the pool have the same Flowcell Type assigned to them.
- Checks that only one pool has been created.
- Changes the value of error and logging messages to reference the type of pool passed as 'bulk' pool.

```
/opt/gls/clarity/bin/java \
-jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar \
script:validate_flowcell_for_input_pools \
-i {stepURI:v2} \
-u {username} \
-p {password} \
-l {compoundOutputFileLuid1} \
-validateSingleOutput true \
-poolType bulk"
```

Calculate Volumes Automation

Triggered when the Calculate Volumes button is selected on the Record Details screen, this automation completes the following actions:

- Calculates the number of samples in the pool:

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: + 1
```

- Sets the value of the Bulk Pool Volume (µl) and PhiX Volume (µl) field, based on the selected Flowcell Type:

```
step.::PhiX Volume (ul):: = !step.hasValue(::% PhiX (0.25nM) Spike-In::) ? 0 :
step.::% PhiX (0.25nM) Spike-In::;
if (input.::Flowcell Type:: == ::SP::) {
step.::Bulk Pool Volume (ul):: = step.'Number of Lanes to Sequence' * 18;
step.::PhiX Volume (ul):: *= 0.7;
};
if (input.::Flowcell Type:: == ::S1::) {
step.::Bulk Pool Volume (ul):: = step.'Number of Lanes to Sequence' * 18;
step.::PhiX Volume (ul):: *= 0.7;
};
if (input.::Flowcell Type:: == ::S2::) {
step.::Bulk Pool Volume (ul):: = step.'Number of Lanes to Sequence' * 22;
step.::PhiX Volume (ul):: *= 0.8;
};
```

```
if (input.::Flowcell Type:: == ::S4::) {
step.::Bulk Pool Volume (ul):: = step.'Number of Lanes to Sequence' * 30;
step.::PhiX Volume (ul):: *= 1.1;
};
if (step.::PhiX Volume (ul):: == 0) {
step.::PhiX Volume (ul):: = ::::;
};
```

- Calculates the Per Sample Volume (µl) to be added to the pool:

```
input.::Per Sample Volume (µl)::= (((input.::Final Loading Concentration (pM)::*
5/ 1000) / input.::Normalized Molarity (nM)::) * step.::Bulk Pool Volume (µl)::)
/ step.::Number of Samples in Pool::
```

> To ensure accurate pipetting of each sample in a pool for sequencing, the Per Sample Volume (µl) value must be equal to or higher than the Minimum Per Sample Volume (µl). The default value (set at 5) can be edited.
>
> Assuming the default Minimum Per Sample Volume (µl) value of 5, completes the following steps for a given batch:
>
> 1. If the smallest Per Sample Volume (µl) value is less than 5, automatically assigns a value of 5 to the sample Adjusted Per Sample Volume (µl) field.
> 2. Adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

- Calculates the Total Sample Volume (µl) field value:

```
step.::Total Sample Volume (µl):: = step.::Total Sample Volume (µl):: +
input.::Adjusted Per Sample Volume (µl)::
```

- If the Total Sample Volume is less than the Bulk Pool Volume, this automation calculates the RSB Volume (µl) field value:

```
if (step.::Total Sample Volume (ul):: >= step.::Bulk Pool Volume (ul)::) {
output.::RSB Volume (ul):: = 0
}
else {
    output.::RSB Volume (ul):: = step.::Bulk Pool Volume (ul):: - step.::Total
    Sample Volume (ul)::
} ;
```

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs:

```
output.::Flowcell Type::= input.::Flowcell Type::;output.::Loading Workflow
Type::= input.::Loading Workflow Type::;
```


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- Uses the `NovaSeq_Xp_Bulk_Pool.csv` and `NovaSeq_Xp_Bulk_Pool2.csv` template files to generate a single CSV file containing information about the bulk pool and the samples it contains. The generated file is available for download in the Files segment of the Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.1) step.

```
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} \
   script:driver_file_generator \
      -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_Bulk_
      Pool.csv \
      -o {compoundOutputFileLuid0}.csv \
   script:driver_file_generator \
      -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_Bulk_
      Pool2.csv \
      -o append.csv \
&& cat append.csv >> {compoundOutputFileLuid0}.csv"
```

- Resets the Total Sample Volume (µl) and Number of Samples in Pool field values so that the automation is idempotent.

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: - 1;
step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: -
input.::Adjusted Per Sample Volume (ul)::
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation completes the following actions:

- Copies the Flowcell Type values from the step inputs to the step outputs.
- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ; nextStep = ::ADVANCE::
```

Master Step Fields

There are seven fields defined on the Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.1) step.

Table 68 Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.1) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
% PhiX (0.25nM) Spike-in	Numeric		Range = 0– 100

For Research Use Only. Not for use in diagnostic procedures.

Field Name	Field Type	Options	Additional Options and Dropdown Items
Bulk Pool Volume (µl) (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 2
Minimum Per Sample Volume (µl)	Numeric	Required Field	Default • 5 Decimal places displayed = 2
Number of Lanes to Sequence	Numeric	Required Field	Decimal places displayed = 0
Number of Samples in Pool (for calculation purposes, not displayed)	Numeric		Default • 0 Decimal places displayed = 0
PhiX Volume (µl) (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 2
Total Sample Volume (µl) (for calculation purposes, not displayed)	Numeric		Default • 0 Decimal places displayed = 0

Global Fields

The following table lists the global custom fields that are configured to display on the Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.1) step.

Table 69 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
RSB Volume (µl)	Numeric	Read Only	Decimal places displayed = 2

For Research Use Only. Not for use in diagnostic procedures.

Field Name	Field Type	Options	Additional Options and Dropdown Items
Flowcell Type	Text Dropdown	Required Field	Presets • SP • S1 • S2 • S4
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User Interaction

Refer to Run Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.1) step section in *NovaSeq 6000Dx Integration v1.1 Validation and Troubleshooting* on page 148.

Step 2: Dilute, Denature and ExAmp (NovaSeqDx v1.1)

In this step, the addition of DPX, NaOH, Tris-HCl, and RSB denatures and dilutes pooled samples. Manually create working pools based on the number of lanes that you want to sequence.

Step input: Bulk pool

Step output: Working pool - variable number (choose how many working pools to create per bulk pool)

Validate Inputs Flowcell Type Automation

Automatically triggered at the beginning of the step, this automation invokes the `validate_flowcell_for_input_pools` script. This script completes the following actions:

- Checks the inputs to the step and validates that the Flowcell Type field is set to a valid value (SP, S1, S2, or S4). This automation also checks that all inputs have the same value for this field.
- The container type selected matches the value in the Flowcell Type field.

```
bash -l -c "/opt/gls/clarity/bin/java \
-jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar \
script:validate_flowcell_for_input_pools \
-i {stepURI:v2} \
-u {username} \
-p {password} \
-l {compoundOutputFileLuid1}"
```


For Research Use Only. Not for use in diagnostic procedures.

Calculate Volumes Automation

Automatically triggered on entry to of the Record Details screen, this automation sets the value of the following fields based on the Flowcell Type:

- BP Aliquot Volume (µl)
- NaOH Volume (µl)
- Tris-HCl Volume (µl)
- DPX1 Volume (µl)
- DPX2 Volume (µl)
- DPX3 Volume (µl)
- Mastermix per Lane (µl)

```
if ( input.::Flowcell Type:: == ::SP:: ) {
output.::BP Aliquot Volume (µl):: = 18 ;
output.::NaOH Volume (µl):: = 4 ;
output.::Tris-HCl Volume (µl):: = 5 ;
step.::DPX1 Volume (µl):: = 126 ;
step.::DPX2 Volume (µl):: = 18 ;
step.::DPX3 Volume (µl):: = 66 ;
output.::Mastermix per Lane (µl):: = 63
};

if (input.::Flowcell Type:: == ::S1::) {
output.::BP Aliquot Volume (µl):: = 18;
output.::NaOH Volume (µl):: = 4 ;
output.::Tris-HCl Volume (µl):: = 5 ;
step.::DPX1 Volume (µl):: = 126;
step.::DPX2 Volume (µl):: = 18;
step.::DPX3 Volume (µl):: = 66;
output.::Mastermix per Lane (µl):: = 63
};
if (input.::Flowcell Type:: == ::S2::) {
output.::BP Aliquot Volume (µl):: = 22;
output.::NaOH Volume (µl):: = 5 ;
output.::Tris-HCl Volume (µl):: = 6 ;
step.::DPX1 Volume (µl):: = 126;
step.::DPX2 Volume (µl):: = 18;
step.::DPX3 Volume (µl):: = 66;
output.::Mastermix per Lane (µl):: = 77
```

```
};
if (input.::Flowcell Type:: == ::S4::) {
output.::BP Aliquot Volume (µl):: = 30;
output.::NaOH Volume (µl):: = 7 ;
output.::Tris-HCl Volume (µl):: = 8 ;
step.::DPX1 Volume (µl):: = 315;
step.::DPX2 Volume (µl):: = 45;
step.::DPX3 Volume (µl):: = 165;
output.::Mastermix per Lane (µl):: = 105
};
```

The automation also completes the following actions:

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs:

```
output.::Flowcell Type::= input.::Flowcell Type::;
output.::Loading Workflow Type::= input.::Loading Workflow Type::;
```

- Uses the `NovaSeq_Xp_Working_Pool.csv` and `NovaSeq_Xp_Working_Pool2.csv` template files to generate a single CSV file. This file contains information about the DPX volume and the volume of BP Aliquot, Mastermix, NaOH, and Tris-HCl to add per working pool. The generated file is available for download in the Files segment of the Dilute, Denature & ExAmp (NovaSeqDx v1.1) step.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} \
   script:driver_file_generator \
      -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_
      Working_Pool.csv \
      -o {compoundOutputFileLuid0}.csv \
   script:driver_file_generator \
      -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_
      Working_Pool2.csv \
      -o append.csv \
&& cat append.csv >> {compoundOutputFileLuid0}.csv"
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation:

- Copies the Flowcell Type values from the step inputs to the step outputs.
- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ; nextStep = ::ADVANCE::
```

Master Step Fields

There are three fields defined on the Dilute, Denature & ExAmp (NovaSeqDx v1.1) step. A script sets these field values. They are not editable while running the step.

The following table lists field configuration details.

Table 70 Dilute, Denature & ExAmp (NovaSeqDx v1.1) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
DPX1 Volume (µl)	Numeric	Read Only	Decimal places displayed = 0
DPX2 Volume (µl)	Numeric	Read Only	Decimal places displayed = 0
DPX3 Volume (µl)	Numeric	Read Only	Decimal places displayed = 0

Global Fields

The following table lists the global custom fields that are configured to display on the Dilute, Denature & ExAmp (NovaSeqDx v1.1) step.

Table 71 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
BP Aliquot Volume (µl)	Numeric	Read Only	• Decimal places displayed = 0
Flowcell Type	Text Dropdown	Required Field	Presets • SP • S1 • S2 • S4

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Field Name	Field Type	Options	Additional Options and Dropdown Items
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
Mastermix per Lane (µl)	Numeric	Read Only	Decimal places displayed = 0
NaOH Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Tris-HCl Volume (µl)	Numeric	Read Only	Decimal places displayed = 2

User Interaction

Refer to Run Dilute, Denature & ExAmp (NovaSeqDx v1.1) step section in *NovaSeq 6000Dx Integration v1.1 Validation and Troubleshooting* on page 148.

Step 3: Load to Flowcell (NovaSeqDx v1.1)

In this step, scan the flow cell barcode into the Clarity LIMS and manually place the working pools into the lanes of the flow cell for the NovaSeqDx run. This step validates the run setup information and generates the sample sheet file.

Step input: Working pool

Step output: Flow cell (output containers: SP, S1, and S2 with 2 lanes, and S4 with 4 lanes)

Validate Inputs and Selected Container Automation

Automatically triggered at the beginning of the step, this automation invokes the validate_flowcell_for_input_pools and validateSelectedContainer scripts. These scripts validate the step inputs and the container selected by the user, as follows.

- Checks that the Flowcell Type field has been set to a valid value (SP, S1, S2, or S4) and that all inputs have the same value.
- Checks that the number of outputs matches the number of lanes on the selected flow cell type. If validation fails, an error message informs that the number of working pools does not match the number of lanes available on the flow cell.

The following table defines the number of samples allowed:

Flow Cell Type	Number of Working Pools
SP	2
S1	2
S2	2
S4	4

- Checks that the container type selected on entry to the Placement screen matches the value in the Flowcell Type field. If validation fails, an error message displays.

```
bash -l -c "/opt/gls/clarity/bin/java \
-jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar
\script:validate_flowcell_for_input_pools \
  -i {stepURI:v2} \
  -u {username} \
  -p {password} \
  -l {compoundOutputFileLuid1} \
  -validateSelectedContainer true"
```

Validate Flowcell Barcode Automation

Automatically triggered on exit of the Placement screen, this automation validates the flow cell barcode scanned into the Clarity LIMS by the user using the following logic:

```
if (input.::Flowcell Type:: == ::SP:: && !output.container.name.matches( ::[A-
Z0-9]{5}DR[A-Z0-9]{2}:: ) ) {
fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: )
};
if (input.::Flowcell Type:: == ::S1:: && !output.container.name.matches( ::[A-
Z0-9]{5}DR[A-Z0-9]{2}:: ) ) {
fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: )
};
if (input.::Flowcell Type:: == ::S2:: && !output.container.name.matches( ::[A-
Z0-9]{5}DM[A-Z0-9]{2}:: ) ) {
fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: )
};
if (input.::Flowcell Type:: == ::S4:: && !output.container.name.matches( ::[A-
Z0-9]{5}DS[A-Z0-9]{2}:: ) ) {
fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: )
};
```

- Copies the Flowcell Type and Loading Workflow Type field values from step inputs to outputs:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ;
```

```
output.::Loading Workflow Type:: = input.::Loading Workflow Type::
```

Validate Run Setup and Generate Sample Sheet Automation

The script definition portion of Validate Run Setup has been moved into the Validation Script custom field due to the 4000 character limitation.

- If modification is required to the Validate Run Setup script, perform the changes directly on Validation Script custom field default value.
- The script definition portion of Generate Sample Sheet is unchanged.

Automatically triggered by selecting a button on the Record Details screen, this automation:

- Copies the Flowcell Type from the step input to the Run Mode field (hidden):

```
step.::Run Mode:: = input.::Flowcell Type::
```

- Validates the parameters entered on the Record Details screen, which is used to set up the run and generate the sample sheet file.
  - Experiment Name only contains alphanumeric, dash, or underscore characters. Spaces are not permitted.
  - When Workflow Type = No Index, both Index Read 1 and Index Read 2 must be 0.
  - When Workflow Type = Single Index, Index Read 1 must be greater than 0 and Index Read 2 must be 0.
  - When Workflow Type = Dual Index, both Index Read 1 and Index Read 2 must be greater than 0.

```
if(!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) {
fail(::Experiment Name contains prohibited characters. Allowed characters are:
a-z, A-Z, 0-9, -, and _::)
};
if (step.::Workflow Type:: == ::No Index::) {
if (step.::Index Read 1:: != 0 || step.::Index Read 2:: != 0) {
fail(::Index Read 1 and Index Read 2 must be 0 if No Index is selected.::)
}
} else if (step.::Workflow Type:: == ::Single Index::) {
if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) {
fail(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if Single
Index is selected.::)
}
} else {
if (step.::Index Read 1:: == 0 || step.::Index Read 2:: == 0) {
fail(::Index Read 1 and Index Read 2 must be greater than 0 if Dual Index is
selected.::)
```


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```
}
};
```

- Checks the Paired End and Read 2 Cycles field values.
  - When Paired End = True, both Read 1 Cycles and Read 2 Cycles must be greater than 0.
  - When Paired End = False, Read 2 Cycles must be 0.

```
if (step.::Paired End::.toBoolean()) {
   if (step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) {
      fail(::Read 1 Cycles and Read 2 Cycles must be greater than 0 if Paired
      End is True.::)
   }
} else {
   if (step.::Read 2 Cycles:: != 0) {
      fail(::Read 2 Cycles must be 0 if Paired End is False.::)
   }
};
```

- Checks the Flowcell field value.
  - If Flowcell Type value is not SP, checks that the value of both Read 1 Cycles and Read 2 Cycles is 151 or less. If the value is greater than 151, an error message displays.

```
if (input.::Flowcell Type::!=::SP:: && step.::Read 1 Cycles:: > 151) {
fail(::Read 1 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
}
if (input.::Flowcell Type::!=::SP:: && step.::Read 2 Cycles:: > 151) {
fail(::Read 2 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
}
```

- Validate Analysis Software Version

```
if (step.hasValue(::Analysis Software Version::) && !step.::Analysis Software
Version::.matches(::^(\\d+)(\\.\\d+)*$::)) {
fail(::Analysis Software Version contains prohibited characters. Allowed
characters are 0-9 and period. It shall start and end with numbers and separated
by single period e.g. 3.8.4::)
};
```

- Checks Override Cycles field value. This field contains only Y, N, I, U, 0–9, and semicolon characters.

```
if (step.hasValue(::Override Cycles::) && !step.::Override Cycles::.matches(::
[YNIU0-9;]+::)) {
fail(::Override Cycles contains prohibited characters. Allowed characters are:
Y, N, I, U, 0-9 and ;. Example: N1Y150;I8;I7N1;Y141U10.::)
};
```

- Sets the next step for samples to ADVANCE, advancing them to the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1) step.

```
nextStep = ::ADVANCE::
```

- Generates the sample sheet and attaches it to the step. For more information, refer to *Sample Sheet Generation* on page 134.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeqDx_BCL2FASTQ_
Samplesheet_v2.csv \
-o {compoundOutputFileLuid0}.csv \
-q true \
-destLIMSID {compoundOutputFileLuid0} \
-l {compoundOutputFileLuid1}
```

Master Step Fields

The following table lists the field configuration details for the fields defined on the Load to Flowcell (NovaSeqDx v1.1) step.

Table 72 Load to Flowcell (NovaSeqDx v1.1) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Analysis Software Version	Text		
BaseSpace Sequence Hub Configuration	Text Dropdown	Required Field	Presets • Not Used • Run Monitoring Only • Run Monitoring and Storage
Custom Recipe Path	Text		
Experiment Name	Text	Required Field	

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Field Name	Field Type	Options	Additional Options and Dropdown Items
Index Read 1	Numeric Dropdown	Required Field Custom Entries	Presets • 0 • 6 • 8 Range = 0–20 Decimal places displayed = 0
Index Read 2	Numeric Dropdown	Required Field Custom Entries	Presets • 0 • 6 • 8 Range = 0–20 Decimal places displayed = 0
Library Tube Barcode	Text	Required Field	
Output Folder	Text	Required Field	
Override Cycles	Text		
Paired End	Text Dropdown	Required Field	Presets • True • False
Read 1 Cycles	Numeric Dropdown	Required Field Custom Entries	Presets • 251* • 151 • 101 • 51 Range = 1–251 Decimal places displayed = 0 *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.

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Field Name	Field Type	Options	Additional Options and Dropdown Items
Read 2 Cycles	Numeric Dropdown	Required Field Custom Entries	Presets • 251* • 151 • 101 • 51 Range = 0–251 Decimal places displayed = 0 *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Run Mode (not displayed in user interface)	Text Dropdown	Read Only	Presets • SP • S1 • S2 • S4
Use Custom Index Read 1 Primer	Toggle Switch		Default • None Set
Use Custom Read 1 Primer	Toggle Switch		Default • None Set
Use Custom Read 2 Primer	Toggle Switch		Default • None Set
Use Custom Recipe	Toggle Switch	Required Field	Default • No

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Field Name	Field Type	Options	Additional Options and Dropdown Items
Validation Script	Multiline Text	Required Field Read Only	Default value provided in the drop-down section following the table. Do not remove this field as it is used by Validate Run Setup and Generate Sample Sheet automation script.
Workflow	Text	Read Only	Default • GenerateFASTQ
Workflow Type	Text Dropdown	Required Field	Presets • No Index • Single Index • Dual Index • Custom

Validation Script

```
step.::Run Mode:: = input.::Flowcell Type::; if (!step.::Experiment
Name::.matches(::[a-zA-Z0-9-_]+::)) { fail(::Experiment Name contains prohibited
characters. Allowed characters are: a-z, A-Z, 0-9, -, and _::) }; if
(step.::Workflow Type:: == ::No Index::) { if (step.::Index Read 1:: != 0 ||
step.::Index Read 2:: != 0) { fail(::Index Read 1 and Index Read 2 must be 0 if
No Index is selected.::) } } else if (step.::Workflow Type:: == ::Single
Index::) { if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) { fail
(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if Single
Index is selected.::) } } else { if (step.::Index Read 1:: == 0 || step.::Index
Read 2:: == 0) { fail(::Index Read 1 and Index Read 2 must be greater than 0 if
Dual Index is selected.::) } }; if (step.::Paired End::.toBoolean()) { if
(step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) { fail(::Read 1
Cycles and Read 2 Cycles must be greater than 0 if Paired End is True.::) } }
else { if (step.::Read 2 Cycles:: != 0) { fail(::Read 2 Cycles must be 0 if
```


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```
Paired End is False.::) }}; if (input.::Flowcell Type:: != ::SP:: && step.::Read
1 Cycles:: > 151) { fail(::Read 1 Cycles must not be larger than 151 if it is
not SPrime Flowcell::) }; if (input.::Flowcell Type:: != ::SP:: && step.::Read 2
Cycles:: > 151) { fail(::Read 2 Cycles must not be larger than 151 if it is not
SPrime Flowcell::) }; if (step.hasValue(::Analysis Software Version::) &&
!step.::Analysis Software Version::.matches(::^(\\d+)(\\.\\d+)*$::)) { fail
(::Analysis Software Version contains prohibited characters. Allowed characters
are 0-9 and period. It shall start and end with numbers and separated by single
period e.g. 3.8.4::) }; if (step.hasValue(::Override Cycles::) &&
!step.::Override Cycles::.matches(::[YNIU0-9;]+::)) { fail(::Override Cycles
contains prohibited characters. Allowed characters are: Y, N, I, U, 0-9 and ;.
Example: N1Y150;I8;I7N1;Y141U10.::) }; step.::Settings Header:: = step.hasValue
(::UMI - Read 1 Length::) || step.hasValue(::UMI - Read 2 Length::) ? ::
[Settings]:: : ::::; nextStep = ::REMOVE::
```

Global Fields

The following table lists the global custom fields that are configured to display on the Load to Flowcell (NovaSeqDx v1.1) step.

Table 73 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Analyte in Derived Sample			
Flowcell Type	Text Dropdown	Required Field	Presets • SP • S1 • S2 • S4
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User Interaction

For more information, refer to the Run Load to Flowcell (NovaSeqDx v1.1) step section in *NovaSeq 6000Dx Integration v1.1 Validation and Troubleshooting* on page 148.

Protocol 4: AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1)

This final protocol contains one fully automated step, AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1).

This step is fully automated. Do not add samples to the Ice Bucket or start the step manually. If steps are manually started, the sequencing service does not update the samples correctly.

Step 1: AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1)

Step input: Library tube from NovaSeqDx Standard or flow cell from NovaSeqDx Xp protocol

Step output: Result file/measurement

In this step, pooled samples are sequenced on the NovaSeq 6000Dx instrument and the run metrics are recorded in Clarity LIMS.

Set Next Steps Automation

If necessary, override the default next step behavior used by the Sequencer API by performing the following steps:

1. Enable the automation and use it to set custom next step behavior and generate a log file. By default, the automation sets the next step to ADVANCE and the samples complete the protocol.
2. Configure the automation to trigger automatically on exit of the Record Details screen.
3. Disable or configure auto-complete behavior by setting the values of the run.autoComplete and autoCompleteOnlyAtSuccess properties. For details, refer to *Configure Autocomplete Properties* on page 146.

Update Lane Number Automation

This automation triggers automatically upon entry to the Record Details screen.

Do not disable or modify this automation to ensure the Lane Number displays properly.

Master Step Fields

The following table shows the master step fields that are configured on the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1) step.

Table 74 Clarity LIMS Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Current Cycle	Numeric	Read Only	Decimal places displayed = 0
Current Read	Numeric	Read Only	Decimal places displayed = 0

Field Name	Field Type	Options	Additional Options and Dropdown Items
Firmware Version	Text	Read Only	
Flow Cell Expiration Date	Date	Read Only	
Flow Cell ID	Text	Read Only	
Flow Cell Lot Number	Text	Read Only	
Flow Cell Mode	Text	Read Only	
Flow Cell Part Number	Text	Read Only	
Flow Cell Side	Text	Read Only	
Instrument Control Software Version	Text	Read Only	
Instrument ID	Text	Read Only	
Instrument Type	Text	Read Only	
Lane Counter	Numeric	Read Only	Default • 1 Hidden
Loading Workflow Type	Text	Read Only	
Output Folder	Text	Read Only	
RTA Version	Text	Read Only	
Run Completion Date	Text	Read Only	Hidden
Run ID	Text	Read Only	
Run Status	Text	Read Only	
Sequencing Log	Multiline Text	Read Only	

Global Fields

The following table shows the global custom fields that capture the run metrics in Clarity LIMS.

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Table 75 Custom Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
% Aligned R1	Numeric	Read Only	Decimal places displayed = 2
% Aligned R2	Numeric	Read Only	Decimal places displayed = 2
% Bases >=Q30 R1	Numeric	Read Only	Decimal places displayed = 2
% Bases >=Q30 R2	Numeric	Read Only	Decimal places displayed = 2
% Error Rate R1	Numeric	Read Only	Decimal places displayed = 2
% Error Rate R2	Numeric	Read Only	Decimal places displayed = 2
% Phasing R1	Numeric	Read Only	Decimal places displayed = 3
% Phasing R2	Numeric	Read Only	Decimal places displayed = 3
% Prephasing R1	Numeric	Read Only	Decimal places displayed = 3
% Prephasing R2	Numeric	Read Only	Decimal places displayed = 3
%PF R1	Numeric	Read Only	Decimal places displayed = 2
%PF R2	Numeric	Read Only	Decimal places displayed = 2
Cluster Density (K/mm^2) R1	Numeric	Read Only	
Cluster Density (K/mm^2) R2	Numeric	Read Only	
Intensity Cycle 1 R1	Numeric	Read Only	
Intensity Cycle 1 R2	Numeric	Read Only	

Field Name	Field Type	Options	Additional Options and Dropdown Items
Reads PF (M) R1	Numeric	Read Only	Decimal places displayed = 2
Reads PF (M) R2	Numeric	Read Only	Decimal places displayed = 2
Yield PF (Gb) R1	Numeric	Read Only	Decimal places displayed = 2
Yield PF (Gb) R2	Numeric	Read Only	Decimal places displayed = 2

Note the following details:

- Values are aggregated across all lanes. Some values (eg, Yield PF (Gb) R1) are summed while others are averaged.
- The names listed previously are the default global custom field names installed with the NovaSeqDx v1.1 configuration provided in the Illumina Preset Protocols (IPP) Package v2.5 or later.
- All global configuration fields are configured on the Container entity.
- All field names are configurable via the Clarity LIMS Configuration > Custom Fields screen (Global Fields tab).
- If field names are changed in Clarity LIMS, they must also be changed through the run.metricUdfNames properties file in the `application.yml` file for the Sequencer API. All fields are configured to be visible on the Record Details screen for the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1) step (in the Sample Details table). For more information, refer to the *application.yml Properties* section of *Components Installed* on page 140.

These global custom fields are configured on the Container entity, visible on each input, and must not be confused with per-lane metrics.

All run metrics tracked in Clarity LIMS are overall metrics for the run. There are currently no per-lane metrics provided by NovaSeq 6000Dx Operating Software.

Sample Sheet Generation

The sample sheet is generated on the step before the run. This step is either Dilute and Denature (NovaSeqDx v1.1) in the NovaSeqDx Standard protocol or Load to Flowcell (NovaSeqDx v1.1) in the NovaSeqDx Xp protocol. This step places samples on the library tube or flow cell that is loaded in the NovaSeq 6000Dx instrument.

In the default configuration, the Validate Run Setup and Generate Sample Sheet automation generates one sample sheet file for use with bcl2fastq v2.20 downstream analysis. This file is in the comma-separated values (CSV) format.

The sample sheet is uploaded to the NovaSeq 6000Dx Operating Software (NVOS) via the `/Illumina/Sequencer/v2/sequencing-run/files` endpoint to the Sequencer API. The file endpoint allows for a file to be downloaded from Clarity LIMS using OAuth (instead of Basic Authentication), which is required for NovaSeq 6000Dx.

The run recipe response sets the sample sheet URL to the link to download the file from this endpoint and sets the sampleSheetRequiresOAuth value to true.

Sample Sheet Content

The following step custom fields that display in the Step Details area of the Record Details screen control the sample sheet content:

- Experiment Name
- Read 1 Cycles
- Read 2 Cycles
- Index 1 Cycles
- Index 2 Cycles
- Analysis Software Version
- Override Cycles

For detailed information on the following items, refer to the bcl2fastq2 *Sample Sheet Generation* section of *Illumina Instrument Sample Sheets (NGS v5.17 & later)* on page 2542.

- Sample sheet generation script parameters and usage
- Sample sheet data and configuration options
- Enabling unique FASTQ file names per sequencing run

NovaSeq 6000Dx Run

The following steps outline the sequence of events that occurs when a flow cell is loaded onto the NovaSeq 6000Dx instrument.

User Authentication and Login

User authentication and login to the NovaSeq 6000Dx instrument is achieved with the getSequencerLoginURL endpoint (`/Illumina/Sequencer/v2/sequencing-run/login`). This endpoint returns the URL that the instrument uses to get an access token.

1. The Sequencer API service provides the NovaSeq 6000Dx Operating Software (NVOS) with a URL for the Clarity LIMS login screen. The NVOS displays this screen to the user. Log in to authorize NVOS to use your account to access Clarity LIMS. This authorization is automatic.

2. Enter credentials:
   - Username and password are passed to the Clarity LIMS server to make sure that the access is valid.
   - By default, the Sequencer API service tries to contact Clarity LIMS at `http://localhost:9080/clarity/`. The installation script sets the URL, but it can be configured with the clarity.url property in the `application.yml` file. For more information, refer to the *application.yml Properties* section of *Components Installed* on page 140.
3. The user is redirected to the Sequencer login page at `/Illumina/Sequencer/v2/sequencer_login`.
   - If authentication is successful, the screen displays a success message. The Clarity LIMS server provides the OAuth token and username by redirecting to `/Illumina/Sequencer/v2/sequencer_login/Success`. This redirect does not reload the page, but allows the NovaSeqDx to continue with run setup.
   - If authentication fails, the login screen does not change and a warning message displays. A background redirect to `/Illumina/Sequencer/v2/sequencer_login/Error` informs the NovaSeq 6000Dx of the failure.

Respond to Validation / Recipe Request Call from Instrument

1. The sequencing-run/recipe/NovaSeqDx endpoint consumes a POST request with a LibraryContainerId, FlowCellId, and reagents information.
   - LibraryContainerId, FlowCellId, and reagents are provided by the NovaSeq 6000Dx.
   - LibraryContainerId and FlowCellId can match either the library tube ID or flow cell ID of the corresponding containers in Clarity LIMS.
   - If there are no containers or multiple containers, an error is generated.
2. All populated containers whose name matches either the LibraryContainerId or FlowCellId are validated as follows.
   - All samples in the container must be queued for a sequencing run step.
   - All samples must be queued for only one sequencing run step.
   - All samples must be queued for the same sequencing run step.

   > Configure the steps that are considered sequencing run steps in the novaseq.sequenceStepNames property in the `application.yml` file. The property is a list and each entry must be the exact name of a sequencing step, not the name of the underlying master step/process type. For more information, refer to the *application.yml Properties* section of *Components Installed* on page 140.
3. After a single container has passed validation, the run setup information (run recipe request) is returned to the NVOS via the API. Then, the run receipt is generated.

Note the following details:

- The run recipe information is read from fields configured on the parent process/master step of the samples in the container. The workflow must include recipe definition in that step.
- By default, the parent process/master step is the Dilute and Denature (NovaSeqDx v1.1) or Load to Flowcell (NovaSeqDx v1.1) step, depending on workflow.
- The default field values match the names of the fields in the NovaSeq 6000Dx v1.1 configuration included in IPP. The field names are configurable through the recipe.udfNames.* properties. Each property corresponds to an entry in the recipe. The value of a property defines the name of the field that is used to populate that entry in the recipe.
- It is assumed that this parent process/master step removes any illegal characters from the Experiment Name field.
- The Clarity LIMS download link to the sample sheet created on the run setup step is used for the SampleSheet value in the recipe. The name of the output of the sample sheet on the setup step is configured through the recipe.sampleSheet.outputName property (Sample Sheet by default) in the `application.yml` file. If no matching output is found, or no file was attached to the output, the value of the recipe.sampleSheet.notAvailableValue property (Not Available by default) is used in the recipe. For more information, refer to the *application.yml Properties* section of *Components Installed* on page 236.

4. After the recipe is generated, it is returned as a *.json file in the response, in the form defined by the Swagger definition for the API.

Run Recipe Contents

The sample sheet contents and the fields configured on the Dilute and Denature (NovaSeqDx v1.1) and Load to Flowcell (NovaSeqDx v1.1) steps control the contents of the run recipe.

Example of run recipe content is as follows.

```
{
"run_name": "NVOS16027_SPrime_2x250",
"run_mode": "S4",
"workflow_type": "DualIndex",
"librarytube_id": "NV0025867-LIB",
"flowcell_id": "",
"sample_loading_type": "NovaSeqStandard",
"rehyb": false,
"paired_end": true,
"read1": 151,
"read2": 151,
"index_read1": 8,
"index_read2": 8,
```

```
"output_folder": "\\\\network_path\\run_data",
"samplesheet": "<path to sample sheet on LIMS server>",
"require_samplesheet_authentication": true,
"usecustomrecipe": false,
"customRecipe": null,
"use_basespace": false,
"basespace_mode": null,
"use_custom_read1_primer": false,
"use_custom_read2_primer": false,
"use_custom_index_read1_primer": false
}
```

Respond to Sequencing Started Call from Instrument

1. Matching container for the run (library tube or flow cell) is found, and contents are validated to be queued for the correct step.

   If no matching container is found, or if the samples in the container are incorrectly queued, an error is thrown and the step is not started. This validation is also done during the run recipe request immediately prior, so this error case should not be encountered.

2. Reagent kits that are configured for the Sequencer API are checked against the configuration for the step to be run:
   - If a kit is configured for the API but does not exist in Clarity LIMS, it is created.
   - If a kit exists, but is not enabled on the step to be run, the step configuration is updated to enable it.
     - If a kit that is to be enabled is Archived, it is set to Active.
     - If a kit that is to be enabled is in Pending status, it is set to Active.
   - If any kits enabled on the step are not configured for the Sequencer API, a warning is logged, and the API cannot complete the step without user intervention.
   - If any kits provided in the recipe request are not configured for the Sequencer API, they are ignored, and a warning is logged during lot tracking.
3. The type of flow cell being used in the run determines the number of replicates for the step.
   - For Standard mode, this information is based on the number of lanes in the flow cell. This information is stored as part of the Sequencer API configuration.
   - For XP mode, there is always one replicate, because there is already one input tracked for each lane.
4. The automated sequencing step is started.

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5. Reagent lots are attached to the step. The Flow Cell and Library Tube reagents in the request are ignored. Information from these reagents is tracked in Clarity LIMS as containers and custom fields. Any other reagents in the request are handled as follows.
   - If the reagent is not configured to be tracked, a warning is logged, and the reagent is ignored.
   - You can look up existing reagent lots by kit name (determined by Sequencer API configuration), name (the serial number in the request), and lot number (the lot number in the request).
   - If one or more matching reagent lots exist in Clarity LIMS, the newest one is used.
   - If the expiry date does not match the expiry in the request, or if the status is not set to Active, the lot is updated to have the correct expiry date and an Active status.

   Otherwise a new lot is created with the following configuration:
   - Reagent Kit—Matching kit as determined from Sequencer API configuration and step 2.
   - Name—Serial number in request.
   - Lot Number—Lot number in request.
   - Expiry—Expiry date in request.
   - Status—Active.

   If any reagents are expected to be in the request but are not, these reagents are logged. This action prevents the step from being completed without user intervention.
6. Master step custom fields are updated with the run information parsed from the request. For more information, refer to *Respond to Record InterOps Metrics Call from Instrument* on page 139.
7. Any important events during this request are logged into the Sequencer API log file and in the Sequencing Log multiline global custom field on the step (assuming the step was started successfully). For more information, refer to *Respond to Record InterOps Metrics Call from Instrument* on page 139.

Respond to Record InterOps Metrics Call from Instrument

When the metrics endpoint receives a POST, it completes the following actions:

- Looks for a matching library tube or flow cell in Clarity LIMS.
- Finds the in-progress sequencing step for that container.

When the step is found, the run metrics in the request are written to container global custom fields in Clarity LIMS.

Respond to Sequencing Completed Call from Instrument

If the sequencing run is successful, after the metrics are recorded, the API call for Sequencing Completed is received. The Sequencing Container matches the Information, and the following occurs in Clarity LIMS:

1. The Current Cycle, Current Read, and Status master step custom fields are updated.

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2. The multiline Sequencing Log master step field is updated.
3. A success message is recorded in the log file at the info level:

```
Sequencing run with Run ID {runId} of Step '{step.name}' ({step.limsid})
completed successfully.
```

4. Clarity LIMS waits for any automations that are triggered automatically as it advances the step.
5. By default, Clarity LIMS assigns the next steps on the outputs to move them on to the first configured next step. If there are no next steps, the protocol is completed.

Respond to Sequencing Errored Call from Instrument

If the sequencing run is unsuccessful, the API call for Sequencing Error is received. The Sequencing Container matches the Information, and the following occurs in Clarity LIMS:

1. The Current Cycle, Current Read, and Status master step custom fields are updated.
2. The multiline Sequencing Log master step field is updated.
3. A failure message is recorded in the log file at the error level:

```
Step '${Step.name}' (${Step.limsid}) failed with Run ID ${runId}.
```

4. The step is left at the Record Details screen.

Respond to Sequencing RunEndedByUser Call from Sequencer

If the user ends a sequencing run, the API call for Sequencing RunEndedByUser is received. The Sequencing Container matches the Information, and the following occurs in Clarity LIMS:

1. The Current Cycle, Current Read, and Status master fields are updated.
2. The multiline Sequencing Log master step field is updated.
3. The following message is recorded in the log file, at the warning level:

```
"Sequencing run with Run ID ${status.runInfo.runId} of Step
'${step.configuration.name}' (${step.limsid}) aborted by user
${status.runInfo.userName}."
```

4. The step is left at the Record Details screen.

Components Installed

The following sections describe the components (files, properties, reagent categories / label groups, reagent kits, and containers) that are installed by default as part of this integration.

Illumina NovaSeq 6000Dx Integration v1.1 is distributed as an RPM package, BaseSpaceLIMS-sequencer-api. This RPM package must be installed on the Clarity LIMS server.

The BaseSpaceLIMS-sequencer-api RPM installs the following items:

- Sequencer API WAR file
- `application.yml` configuration file
- The following configuration scripts:

- `configure_sequencer_api_proxy.sh`
- `configure_sequencer_api_env.sh`
- `configure_sequencer_api_application.sh`

The NovaSeq 6000Dx v1.1 configuration is delivered through the Illumina Preset Protocols (IPP) Package v2.5. IPP Package v2.5 requires installation of NGS Extensions Package v5.22.0 or later.

- If the NGS Extensions Package is not already installed, or if a version earlier than v5.22.0 is installed, the latest version is installed by default with the NovaSeq 6000Dx integration. For details, refer to *NovaSeq 6000Dx Integration v1.1 Release Notes* on page 184.
- If NGS Extensions Package v5.22.0 or later is already installed, upgrade is not forced or required.

NovaSeq 6000Dx Integration RPM Components

The following table lists the components installed by the RPM package.

Files Installed	Location and Description
`Illumina#Sequencer#v2.war`	`/opt/gls/clarity/tomcat/current/webapps` War file for NovaSeqDx v1.1 API.
`configure_sequencer_api_application.sh`	`/opt/gls/clarity/config/` Script that configures the Sequencer API application through its external `application.yml` file.
`configure_sequencer_api_env.sh`	`/opt/gls/clarity/config/` Script that configures the Clarity LIMS Tomcat configuration to include Secret Util settings.
`configure_sequencer_api_proxy.sh`	`/opt/gls/clarity/config/` Script that configures the proxy to allow communication with the Sequencer API (NovaSeqDx v1.1 API) application.
`sequencer-api.conf`	`/etc/httpd/clarity/` Proxy setting to communicate with Clarity LIMS.

`application.yml` Properties

The `application.yml` file is at `opt/gls/clarity/extensions/sequencing-api/`.

- All properties are configured automatically during installation. You can also configure properties after installation, by editing the `application.yml` file. If you make changes to the file, you must restart Tomcat.

- File properties represent all custom field names, both for recipe and run information.
- For all custom fields, the names (values in this file) can be changed. However, the property names cannot be changed, removed, or added to. For example, the Loading Workflow Type can be changed, but recipe.udfNames.sampleLoadingType cannot.

Table 76 `application.yml` Properties Installed

Property	Description
spring.profiles.active	Tells the application that it is running deployed in Tomcat. Default value: tomcat (Do not change)
clarity.url	Base URL that the Sequencer API service uses to contact the Clarity LIMS. The installation script (configure_sequencer_api_application) prompts for this property.
clarity.username	Username to be used when communicating with Clarity LIMS.
security.signing-key	Private key that is used when signing/validating OAuth tokens. Changing this property invalidates any issued tokens.
security.token-expiry	Specifies (in hours) the expiry period for login tokens issued by the Sequencer API. Default value: 88
novaseq.sequenceStepNames	List of NovaSeq 6000Dx sequencing run step names for which the integration can find samples queued. • Names must be an exact match to the name of the step in Clarity LIMS (not the master step/process type). • Each line is indented to the same point and start with a dash (-). Default - AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7) - AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1)

Property	Description

novaseq.flowcells	List of supported NovaSeq 6000Dx flow cell types.
	• Type names exactly match the values used by the NVOS run status requests.
	• To add more types, provide both type and lanes properties, indented to the same point and starting with a dash (-).
	Default value:
	`- type: "SP"`
	`lanes: 2`
	`- type: "S1"`
	`lanes: 2`
	`- type: "S2"`
	`lanes: 2`
	`- type: "S4"`
	`lanes: 4`

novaseq.reagents	List of supported reagent kits to be tracked on sequencing steps.
	- lotName: The name of the lot as it appears in the run started request.
	- kitName: The name of the corresponding reagent kit in Clarity LIMS that is used for lot tracking.
	• The name can differ in the NVOS request versus the Clarity LIMS configuration.
	Default value:
	`- lotName: "Buffer"`
	`kitName: "Buffer Cartridge"`
	`- lotName: "Cluster"`
	`kitName: "Cluster Cartridge"`
	`- lotName: "SBS"`
	`kitName: "SBS Cartridge"`

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Property	Description
recipe.udfNames	Configures the names of the fields used in the run recipe. For more information, refer to *Run Recipe Contents* section in *NovaSeq 6000Dx Run* on page 135. • Each value must be enclosed in quotes. Example: recipe.udfNames.runMode "Run Mode" • Value provided for sampleLoadingType must match the value provided in the run configuration. Example: recipe.udfNames.sampleLoadingType "Loading Workflow Type"
recipe.sampleSheet.outputName recipe.sampleSheet.notAvailableValue	Configures the name of the sample sheet file placeholder on the step where run recipe information is populated, and the property value to use when the file is not found.
run.stepUdfNames	Configures the master step field names to be used when recording run results. For more information, refer to the *Master Step Fields* section in *NovaSeq Run* on page 583 • Each value must be enclosed in quotes. Example: run.stepUdfNames.runID "Run ID" • Value provided for sampleLoadingType must match the value provided in the run configuration. • Example: run.stepUdfNames.sampleLoadingType "Loading Workflow Type". It is expected that the run step field and the field on the input derived sample share a name.
run.metricUdfNames	Configures the result file global custom field names to be used when recording run results. These are the fields that capture the run data. For more information, refer to *Global Custom Fields* section.
run.autoComplete	Determines whether the sequencing run step is completed automatically after parsing the run metrics on a run (refer to run.autoCompleteOnlyAtSuccess). Default value: true For more information, refer to the *Configure Autocomplete Properties* on page 146 section.

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Property	Description
run.autoCompleteOnlyAtSuccess	Determines which of the following options are applicable to the sequencing run step: • Autocompleted when the run status is RunCompletedSuccessfully (if autoCompleteOnlyAtSuccess is true) • Autocompleted regardless of the run status (if autoCompleteOnlyAtSuccess is false). Default value: true For more information, refer to the *Configure Autocomplete Properties* on page 146 section.

Reagent Categories/Label Groups

- TruSeq HT Adapters v2 (D7-D5)

Reagent Kits

- Buffer Cartridge
- Cluster Cartridge
- DPX1
- DPX2
- DPX3
- NaOH
- Resuspension Buffer (RSB)
- SBS Cartridge
- Tris-HCl

Container Types

- Library Tube
- SP
- S1
- S2
- S4

This integration supports the following items:

- Library tube with barcode provided in the following formats:

  - RUO mode: [A-Z]{2}[0-9]{7}-[A-Z]{3}
    Example: AB1234567-LIB
  - DX mode: DX[0-9]{7}-[A-Z]{3}
    Example: DX1234567-LIB
- SP, S1, S2, or S4 flow cell with barcode provided in one of the following formats:
  - SP and S1 flow cell: [A-Z0-9]{5}DR[A-Z0-9]{2}
  - S2 flow cell: [A-Z0-9]{5}DM[A-Z0-9]{2}
  - S4 flow cell: [A-Z0-9]{5}DS[A-Z0-9]{2}
    Example: H1991DMXX

NVOS Configuration

For details on configuring the NovaSeq 6000Dx Operating Software (NVOS) for integration with Clarity LIMS, contact the Clarity LIMS Support team.

Routing Script Requirements

The requirements for the routing script functionality are as follows.

- On the steps that use the routing script (Define Run Format (NovaSeqDx v1.1) and Dilute and Denature (NovaSeqDx v1.1)):
  - The Next Step for all samples must be set to Remove from workflow. A script sets this value. The value must not change in the Assign Next Steps screen.
- In the protocol configuration screen, the following settings are required and must not be changed:
  - In the Next Steps section, for the last step in the protocol, the method of assigning the next step must be set to Automatic.

Configure Autocomplete Properties

The `application.yml` configuration file contains l the following properties:

- run.autoCompleteOnlyAtSuccess
- run.autoComplete

These properties determine the conditions under which the last step of the NovaSeq 6000Dx workflow, AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1), is automatically completed. The two scenarios for autocompletion of the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1) step are as follows.

- The step autocompletes only when the run status is RunCompletedSuccesfully (default behavior).
- The step autocompletes regardless of the run status.

By default, both properties are set to true and the step only completes if the run is successful. If the run fails or is aborted, the step must be completed manually in Clarity LIMS.

For the step to autocomplete regardless of the result of the sequencing run, change the autoCompleteOnlyAtSuccess property value to false.

Enable/Disable autoComplete Properties

1. Open the `application.yml` file at `/opt/gls/clarity/extensions/sequencer-api/application.yml`:

```
vi /opt/gls/clarity/extensions/sequencer-api/application.yml
```

2. Edit the value of the autoCompleteOnlyAtSuccess property as required.
3. Save the file.

autoComplete Properties

The following table shows how the combined value of the run.autoComplete and run.autoCompleteOnlyAtSuccess properties affects the autoComplete behavior of the sequencing step.

Table 77 run.autoComplete and run.autoCompleteOnlyAtSuccess Value Matrix

run.autoComplete property value	run.autoCompleteOnlyAtSuccess property value	Outcome
true	true	• Step automatically completes only if the sequencing run is successful. • If run is not successful, the step does not automatically complete, and the run details are recorded in Clarity LIMS.
true	false	• Step automatically completes, regardless of the run status.
false	true	• Step does not automatically complete. • Run details are recorded in Clarity LIMS.
false	false	

Rules and Constraints

- The workflow configuration contains several validation checks. To make sure that the calculations work properly, it is important that you do not disable any of this validation logic. The validation checks determine the following information:

  - Which samples, and how many, can enter each step together.
  - Which samples, and how many, can be pooled together.
- The library tube ID must be unique. There must not be multiple library tube containers in the system with the same name.
- Reagent labels (indexes) must be unique.
- Only controls are permitted as unindexed samples. All other unindexed samples and pools are not permitted.
- For sample sheet generation constraints, refer to the *Bcl2fastq2 Sample Sheet Generation* section in *Illumina Instrument Sample Sheets (NGS v5.17 & later)* on page 2542.
- Do not manually start the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1) step. This step is fully automated and the sequencing service does not update samples correctly if they have been manually started.
- For the automated run to start successfully, the Validate Run Setup and Generate Sample Sheet button must be selected.

NovaSeq 6000Dx Integration v1.1 Validation and Troubleshooting

This section explains how to validate the installation of the Illumina NovaSeqDx Integration Package v1.1. The integration only supports Clarity LIMS v5.1 and later.

The validation process involves the following items:

- Running samples through the Library Prep Validation workflow.
  - The workflow contains a single-step protocol that models the library prep required to produce normalized libraries. At the end of the step, the normalized libraries advance to the workflow selected.
- Running normalized libraries through the NovaSeqDx v1.1 workflow validates the following items:
  - Successful routing of samples from the Run Format (NovaSeqDx v1.1) step to the NovaSeqDx Standard (NovaSeqDx v1.1) or NovaSeqDx Xp (NovaSeqDx v1.1) step.
  - Automated generation of v2 sample sheet. This file automatically uploads to the sequencing system via the Sequencer API.
  - Automatic validation of run setup information. Information is uploaded to the NovaSeq 6000Dx Operating Software (NVOS) via the Sequencer API and is used to create the run recipe and initiate the run.
  - Automated tracking of the NovaSeq 6000Dx sequencing run and parsing of run statistics into Clarity LIMS, via the Sequencer API.

The validation steps assume that the NovaSeqDx Integration Package v1.1 is installed and you have imported the default Clarity LIMS configuration.

Activate Workflow, Create Project, Add and Assign Samples

The following steps set up Clarity LIMS in preparation for running samples through the Library Prep Validation v2.3.0 and NovaSeqDx v1.1 workflows.

1. On the Configuration tab, under Workflows, activate both the Library Prep Validation v2.3.0 and NovaSeqDx v1.1 workflows.
2. On the Projects and Samples screen, create a project and add samples to it.
3. Assign the samples to the Library Prep Validation workflow.

Library Prep Protocol: Library Prep Validation v2.3.0

This single-step protocol models the library prep required to produce normalized libraries that are ready for the NovaSeqDx v1.1 workflow.

Run Library Prep Validation v2.3.0

1. In Lab View, locate the Library Prep Validation v2.3.0 protocol.
   Samples are queued for the Library Prep Validation v2.3.0 step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Placement screen, complete the following actions:
   a. In the Placed Samples area on the right, in the Container field, scan or enter the barcode of the destination container.
   b. Select the samples from the Samples to be Placed area on the left, and drag them to the container wells on the right.
   c. Select Add Labels.
5. On the Add Labels screen, the Labels to Add list is populated with labels from the TruSeq HT Adapters v2 (D7-D5) label group. The group is the only label group configured in the integration and is used by default in this step.
   a. Select labels on the left, and drag them to the container on the right to assign them to placed samples.
   b. Select Record Details.
6. On the Record Details screen, complete the following actions:
   a. In the Sample Details table, enter values for Normalized Molarity. Type values individually or copy and paste to enter multiple values from a spreadsheet.
   b. The Sequencing Instrument column indicates the instrument to be used for the sequencing run. Select NovaSeqDx.
   c. Populate the fields in this column using one of the following methods:
      - To populate fields individually for each sample, select from the drop-down list in each row.
      - To populate all fields at one time, copy and paste from a spreadsheet.

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Alternatively, select inside the table and press Ctrl + A to select all rows. At the top of the table, select Sequencing Instrument and then select NovaSeqDx from the adjacent drop-down list. Select Apply.

If working with many samples, copy and paste to insert multiple values from a spreadsheet as follows.

a. Select Copy Samples to copy the sample information from Clarity LIMS to the Clipboard.
b. Open a blank Excel document and paste the contents of the clipboard into the spreadsheet.
c. In the spreadsheet, enter the Molarity (nM) and Sequencing Instrument values. Select the entire spreadsheet including the headers (Ctrl + A), and copy (Ctrl + C) the information to the Clipboard.
d. Within Clarity LIMS, select the Paste Samples button. Paste the contents of the clipboard (Ctrl + V) into the Update Table window and then select Update to paste the contents into the Sample Details table.

7. Select Next Steps.
   Triggers the Next Steps automation, which sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step.
8. On the Assign Next Steps screen in the Samples table, the Next Step value for all samples is prepopulated with Remove from workflow.

   Do not change this value. If Next Step is not set to Remove from workflow, the routing script does not route samples correctly.

9. Select Finish Step.
   On exit from the step, the Routing Script automation is triggered. This automation assigns samples to the first step of the NovaSeqDx v1.1 workflow, Define Run Format (NovaSeqDx v1.1). This is the only step in Protocol 1: Run Format (NovaSeqDx v1.1).

Protocol 1: Run Format (NovaSeqDx v1.1)

This protocol includes a single step, Define Run Format (NovaSeqDx v1.1). The step allows for the assignment of per sample values for the following fields:

- NovaSeqDx Run Mode
- Loading Workflow Type
- Normalized Molarity
- Flowcell Type
- Final Loading Concentration (pM)

At the end of the step, samples are routed to the NovaSeqDx Standard or NovaSeqDx Xp protocol. This routing is done according to the selected Loading Workflow Type. The selected NovaSeqDx Run Mode must be compatible with the Loading Workflow Type and Flowcell Type.

Step 1: Run Define Run Format (NovaSeqDx v1.1)

1. In Lab View, locate the Run Format (NovaSeqDx v1.1) protocol. The samples are queued for the Define Run Format (NovaSeqDx v1.1) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Record Details screen in the Sample Details table, populate the following fields (values can vary across samples):
   - NovaSeqDx Run Mode—Select DX or RUO.
   - Loading Workflow Type—Select NovaSeq Standard or NovaSeq Xp from the drop-down list.
   - Flowcell Type—Select SP, S1, S2, or S4.
   - Final Loading Concentration (pM)—Select from the two preset options: 225 (for PCR-free workflows), 400 (for Nano workflows), or enter a different value.
   - Normalized Molarity (nM)—These values are copied over from the previous step. If this column is not populated during library prep, enter the values here.
5. Select Next Steps, which triggers the Set Next Steps automation and the following actions:
   - Sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step.
   - Checks that the NovaSeqDx Run Mode value is compatible with the Loading Workflow Type and Flowcell Type. This automation also generates an error message if the Loading Workflow Type or the Flowcell Type are incompatible.
   - Calculates the Minimum Molarity.
   - Checks Normalized Molarity value. For samples with no Normalized Molarity value (eg, empty value, not including 0), generates an error message notifying that the field cannot be empty.
   - Compares each sample Normalized Molarity value with the Minimum Molarity value.
6. On the Assign Next Steps screen:
   - In the Sample Details table, the Next Step for all samples is prepopulated with Remove from workflow, regardless of the Loading Workflow Type.

     Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not routed samples correctly.

   - For samples whose Normalized Molarity value is lower than the Minimum Molarity value, the Loading Workflow Type is set to Remove from workflow. A message is recorded in the Warning field for the sample.
     To proceed, choose one of the following options:

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- Return to the Record Details screen and adjust the Normalized Molarity value so that it equals or exceeds the Minimum Molarity value. The Loading Workflow Type must be set to NovaSeq Standard or NovaSeq Xp, as applicable.
- Complete the protocol without correcting the Normalized Molarity value. In this case, the samples in question are removed from the Clarity LIMS workflow.

7. Select Finish Step. The Routing Script automation is triggered:
   - Samples whose Loading Workflow Type is set to Remove from workflow are removed from the Clarity LIMS workflow. (Occurs when the Normalized Molarity value is lower than the Minimum Molarity.)
   - Samples whose Loading Workflow Type is set to NovaSeq Standard are routed to the Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.1) step. This step is the first step in the NovaSeq Standard protocol.
   - Samples whose Loading Workflow Type is set to NovaSeq Xp are routed to the Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.1) step. This step is the first step in the NovaSeq Xp protocol.

Protocol 2: NovaSeqDx Standard (NovaSeqDx v1.1)

In this protocol, samples are pooled and added to the library tube in preparation for the NovaSeq 6000Dx run. The protocol contains two steps:

1. Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.1)
2. Dilute and Denature (NovaSeqDx v1.1)

Step 1: Run Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.1)

1. In Lab View, locate the NovaSeqDx Standard (NovaSeqDx v1.1) protocol.
   Samples for the Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.1) step are queued.
2. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
   When the step begins, the Validate Single NovaSeqDx Run Mode automation is triggered. This automation checks that the samples have the same NovaSeqDx Run Mode (RUO or DX).
4. On the Pooling screen, perform the following actions:
   a. Create a pool by dragging samples into the Pool Creator.

      Only create one pool.

   b. Enter a name for the pool or accept the default name (eg, Pool #1).
   c. Select Record Details.
      After exiting the Pooling screen, the Validate Inputs Flowcell Type and Single Pool is triggered. The automation verifies the following items:

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- All samples in the pool have been assigned the same Flowcell Type.
- Only one pool has been created.

5. On the Record Details screen in the Step Details area, complete the two required fields and one optional field, as needed:
   - Number of Flowcells to Sequence—Used in volume calculations, to make sure that the volumes are sufficient for the number of times the pool is sequenced.
   - Minimum Per Sample Volume (µl)—Used to calculate how much of each sample is included in the pool. The field is prepopulated with the configured default value 5 µl, but can be edited.
   - % PhiX (2.5 nM) Spike-In—Used to calculate the volume of PhiX v3 control included in the pool for the given percentage of spike-in. The field is optional and can be edited.

     Assuming the default Minimum Per Sample Volume (µl) value of 5, for a given batch:
     - If the smallest Per Sample Volume (µl) value is < 5, Clarity LIMS automatically assigns a value of 5 to the Adjusted Per Sample Volume (µl) field.
     - Clarity LIMS changes the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.
6. In the Sample Details table, select the pool icon to view details on the pool composition.
7. Select Calculate Volumes to trigger the Calculate Volumes automation. This automation performs calculations based on the selected Flowcell Type.
   - Calculates Bulk Pool Volume (µl) value.
   - Sets the value of the Bulk Pool Volume (µl) field.
   - Calculates the PhiX Volume (µl) value if % PhiX (2.5 nM) Spike-In has value.
   - Calculates the Per Sample Volume (µl) value to be added to the pool.
   - Calculates the Total Sample Volume (µl) value.
   - If the Total Sample Volume is less than the Bulk Pool Volume, it calculates the RSB Volume (µl) value.
   - Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
   - Populates the Volume of Pool to Denature (µl), NaOH Volume (µl) and Tris-HCl Volume (µl) columns of the Sample Details table. The script sets the values, and is not editable.
   - The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains volume information about the pool and the individual samples that it contains. Select the file to download it and then open it in Excel.
8. Select Next Steps to trigger the Set Next Step automation. This automation sets the next step for samples to ADVANCE and advances them to the next step in the protocol, Dilute, and Denature (NovaSeqDx v1.1). The automation also copies the NovaSeqDx Run Mode value from the input pool to the output pool.

On the Assign Next Steps screen, the next step for samples is set to the next step in the workflow: Dilute and Denature (NovaSeqDx v1.1).

9. Select Finish Step.
   At the end of this step, the pool of samples automatically advances to the Dilute and Denature (NovaSeqDx v1.1) step.

Step 2: Run Dilute and Denature (NovaSeqDx v1.1)

1. In Lab View, locate the NovaSeqDx Standard (NovaSeqDx v1.1) protocol. The pool of samples queued for the Dilute and Denature (NovaSeqDx v1.1) step are listed.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work. The Validate Single Input automation is triggered. This automation verifies that only one container is input for the step.
4. On the Placement screen:
   a. Drag the pool into the library tube in the Placed Samples area.
   b. Scan or type the barcode of the library tube into the Library Tube field.
   c. Select Record Details.
   After exiting the Placement screen, the Validate Library Tube Barcode automation makes sure that the library tube barcode conforms to the barcode mask that corresponds with the NovaSeqDx Run Mode in the following table. If the barcode does not match, an error message displays. The automation also copies the Flowcell Type and Loading Workflow Type values from step inputs to outputs.

NovaSeqDx Run Mode	Valid Barcode Mask
RUO	[A-Z]{2}[0-9]{7}-[A-Z]{3}
DX	DX[0-9]{7}-[A-Z]{3}

5. On the Record Details screen, the Reagent Lot Tracking section lets you tracks the NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. Add and activate lots for these reagents as follows.
   a. In a new browser tab, open an additional instance of Clarity LIMS.
   b. Navigate to the Consumables configuration screen.
   c. Select the NaOH reagent kit and select New Lot.
   d. Enter the lot details.
   e. Set the Status of Reagent Lot toggle switch to Active.
   f. Select Save.
   g. Repeat this process to add and activate Resuspension Buffer and Tris-HCl reagent lots.
   h. Return to your original working browser tab and refresh the page.

6. In the Reagent Lot Tracking section, select from the active lots displayed in each drop-down list. The fields displayed on the Record Details screen are used to set up the run and generate the sample sheet. Some of these fields are autopopulated and some must be completed manually.

Table 78 Fields Displayed on Record Details Screen of Dilute and Denature (NovaSeqDx v1.1)

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.
BaseSpace Sequence Hub Configuration	Presets • Not Used • Run Monitoring Only • Run Monitoring and Storage
Workflow	Automatically populated with the preset GenerateFASTQ.
Workflow Type	Presets • No Index • Single Index • Dual Index • Custom
Index Read 1	Presets • 0 • 6 • 8 • Type a value between 0 and 20
Index Read 2	Presets • 0 • 6 • 8 • Type a value between 0 and 20
Paired End	Presets • True • False

Field	Value
Read 1 Cycles	Presets • 251 • 151 • 101 • 51 • Type a value between 1 and 251 *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Read 2 Cycles	Presets • 251 • 151 • 101 • 51 • Type a value between 0 and 251 *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Use Custom Read 1 Primer	Select if applicable.
Use Custom Read 2 Primer	Select if applicable.
Use Custom Index Read 1 Primer	Select if applicable.
Use Custom Recipe	Select if applicable.
Custom Recipe Path	If the Use Custom Recipe option is selected, enter the path to the custom recipe file to be used.
Output Folder	Enter network path for sequencing run folder. For example: `\\networkshare\run_data`
Analysis Software Version	Enter the software version for BCL Convert Analysis for v2 sample sheet. Leave blank if not applicable.
Override Cycles	Enter the Override Cycles value for v2 sample sheet. Leave blank if not applicable.

7. On the Record Details screen, select Validate Run Setup and Generate Sample Sheet. This triggers the automation script, which performs the following actions:
   - Validates the parameters entered on the Record Details screen.
   - Generates the sample sheet and attaches it to the placeholder in the Files area of the Record Details screen.

> The v2 sample sheet generated does not include Cloud and Analysis sections. Contact Illumina Support for information on generating v2 sample sheets with those sections.

- Select Next Steps.

8. On the Assign Next Steps screen, the Next Step for samples is prepopulated with Remove from workflow. The Routing Script automation expects this value, and requires the value to advance samples to the next step.

   > Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.

9. Select Finish Step.
   After exiting the step, the following actions occur:
   - The Routing Script automation is triggered and samples are routed to AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1).
   - In Lab View, the pool of samples is queued for the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1) step.

   At this point in the workflow, the user interaction ends. Proceed to AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1).

   > Do not add samples to the Ice Bucket or start the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1) step. The integration adds samples automatically.

Protocol 3: NovaSeqDx Xp (NovaSeqDx v1.1)

In this protocol, samples are pooled and added to lanes on the NovaSeq 6000Dx flow cell. The option selected in the Define Run Format (NovaSeqDx v1.1) step determines the flow cell type.

The protocol contains three steps:

1. Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.1)
2. Dilute, Denature & ExAmp (NovaSeqDx v1.1)
3. Load to Flowcell (NovaSeqDx v1.1)

Step 1: Run Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.1)

1. In Lab View, locate the NovaSeqDx Xp (NovaSeqDx v1.1) protocol. The samples are queued for the Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.1) step.
2. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Pooling screen, do as follows.
   a. Create a pool by dragging samples into the Pool Creator.

      > Create only one pool.

b. Enter a name for the pool or accept the default name (eg, Pool #1).

c. Select Record Details.

5. On exiting the Pooling screen, the Validate Inputs Flowcell Type and Single Pool automation is triggered. The automation verifies the following items:
   - All samples in the pool are assigned the same Flowcell Type.
   - Only one pool was created.
6. On the Record Details screen, in the Step Details area, complete the two required fields and one optional field, as needed:
   - Number of Lanes to Sequence—Used in volume calculations to make sure that volumes are sufficient for the number of times the pool is sequenced.
   - Minimum Per Sample Volume (µl)—Used to calculate how much of each sample is included in the pool. The field is prepopulated with the configured default value 5 µl, but can be edited.
   - % PhiX (0.25nM) Spike-In—Used to calculate the volume of PhiX v3 control to be included in the pool for the given percentage of spike-in. The field is optional and can be edited.

     Assuming the default Minimum Per Sample Volume (µl) value of 5, for a given batch:
     - If the smallest Per Sample Volume (µl) value is < 5, Clarity LIMS automatically assigns a value of 5 to the samples Adjusted Per Sample Volume (µl) field.
     - Clarity LIMS then adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.
7. [Optional] In the Sample Details table, select the pool icon to view details on the pool composition.
8. Select Calculate Volumes to trigger the Calculate Volumes automation. This automation performs volume calculations based on the selected Flowcell Type, as follows.
   - Calculates Bulk Pool Volume (µl) value.
   - Calculates the Per Sample Volume (µl) value to be added to the pool.
   - Calculates PhiX Volume (µl) value if % PhiX (0.25nM) Spike-In has value.
   - Calculates the Total Sample Volume (µl) value.
   - If the Total Sample Volume is less than the Bulk Pool Volume, the automation calculates the RSB Volume (µl) value.
   - Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
   - Generates the Calculation File (CSV) and attaches it to the step. This file contains volume information about the samples and RSB buffer to add to the pool. Select the file to download it, and then open it in Excel.
9. Select Next Steps to trigger the Set Next Step automation. This automation performs the following actions:

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- Copies the Flowcell Type values from the step inputs to the step outputs.
- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol—Dilute, Denature & ExAmp (NovaSeqDx v1.1).

On the Assign Next Steps screen, the next step for samples is set to Dilute, Denature & ExAmp (NovaSeqDx v1.1).

10. Select Finish Step.
    At the end of this step, the pool of samples automatically advances to the Dilute, Denature & ExAmp (NovaSeqDx v1.1) step.

Step 2: Run Dilute, Denature & ExAmp (NovaSeqDx v1.1)

1. In Lab View, locate the NovaSeqDx Xp (NovaSeqDx v1.1) protocol. The pool of samples queued for the Dilute, Denature & ExAmp (NovaSeqDx v1.1) step displays.
2. Add the pool to the Ice Bucket and select View Ice Bucket.
3. [Optional] On the Ice Bucket screen, set the number of derivatives to create (placed into the flow cell lanes) and select Begin Work.
4. On entry to the Record Details screen, the Calculate Volumes automation is triggered.
   This automation sets the following values based on the selected Flowcell Type:
   - BP Aliquot Volume (µl)
   - NaOH Volume (µl)
   - Tris-HCl Volume (µl)
   - DPX1 Volume (µl)
   - DPX2 Volume (µl)
   - DPX3 Volume (µl)
   - Mastermix per Lane (µl)
   - The automation also populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
   - The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains information about the DPX Mastermix volume and the volume of Mastermix, NaOH, and Tris-HCl to add per working pool (refer to the next step).
5. On the Record Details screen, the Reagent Lot Tracking section tracks the DPX1, DPX2, DPX3, NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. These reagents must be added.
   To add and activate reagent lots, do as follows.
   a. In a new browser tab, open an additional instance of Clarity LIMS.
   b. Select one of the reagent kits previously listed and select New Lot.

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c. Enter the lot details.
d. Set the Status of Reagent Lot toggle switch to Active.
e. Select Save.
f. Repeat this process to add and activate the remaining reagent lots.
g. Return to your original working browser tab and refresh the page.

6. Add and activate reagent lots as follows.
   On the Record Details screen, the Reagent Lot Tracking section tracks the reagents used in the step.
   a. In a new browser tab, open another instance of Clarity LIMS.
   b. Navigate to the Consumables screen.
   c. Select one of the following reagent kits:
      - DPX1
      - DPX2
      - DPX3
      - NaOH
      - Resuspension buffer
      - Tris-HCl
   d. Select New Lot.
   e. Enter the lot details.
   f. Set the Status of Reagent Lot toggle switch to Active.
   g. Select Save.
   h. Repeat steps c–g for the remaining reagent lots.
   i. Return to the original instance of Clarity LIMS and refresh.
7. On the Record Details screen, perform the following actions:
   a. In the Reagent Lot Tracking section, select from the active lots displayed in each drop-down list.
   b. In the Step Details section, verify that the DPX1, DPX2, and DPX3 reagent volume values are populated. The script sets these values and they cannot be edited.
   c. [Optional] In the Sample Details table, select the pool icon to view details of the working pool composition.
   d. Make sure that the following values and columns are populated:
      - BP Aliquot
      - Mastermix per lane
      - NaOH
      - Tris-HCl

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- Flowcell Type
- Loading Workflow Type

A script sets these values and columns and they cannot be edited. The working pool number is appended to the bulk pool name, so that you can identify which working pools are derived from the same bulk pool.

8. In the Files area, select the Calculation File (CSV) to open it and view details on the DPX Mastermix volume and the volume of Mastermix, NaOH, and Tris-HCl to add per working pool.
9. Select Next Steps.
   On the Assign Next Steps screen, the next step is already set to Load to Flowcell (NovaSeqDx v1.1).
10. Select Finish Step.

Step 3: Run Load to Flowcell (NovaSeqDx v1.1)

1. On the Ice Bucket screen, perform the following actions:
   a. In the Container Options panel, select the appropriate flow cell type from the Destination Container drop-down list.
   b. Select Begin Work.
2. The Validate Inputs and Selected Container automation checks the following information:
   - The Flowcell Type field is set to a valid value (SP, S1, S2, or S4) and each input has the same value for the field.
   - The container type selected matches the value in the Flowcell Type field.
   - The number of outputs matches the number of lanes on the selected flow cell type. If validation fails, an error message reports the number of working pools does not match the number of lanes available on the flow cell.
3. On the Placement screen, do as follows.
   a. Drag pools from the left of the screen over into the Placed Samples area on the right.
   b. Scan or type the barcode of the flow cell into the Flowcell field.
   c. Select Record Details.

   After exiting the Placement screen, the Validate Flowcell Barcode automation makes sure that the container barcode conforms to the barcode mask for the selected Flowcell Type. This automation also copies the Flowcell Type and Loading Workflow Type field values from step inputs to outputs. The following table shows the barcode mask information.

Flowcell Type	Barcode Mask
SP	[A-Z0-9]{5}DR[A-Z0-9]{2}
S1	[A-Z0-9]{5}DR[A-Z0-9]{2}

Flowcell Type	Barcode Mask
S2	[A-Z0-9]{5}DM[A-Z0-9]{2}
S4	[A-Z0-9]{5}DS[A-Z0-9]{2}

4. The fields displayed on the Record Details screen are used to set up the run and generate the sample sheet.
   Some of these fields are autopopulated and some must be entered manually. Refer to the following table for details.

   Table 79 Fields Displayed on Record Details Screen of Load to Flowcell (NovaSeqDx v1.1) Step

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. No spaces.
BaseSpace Sequence Hub Configuration	Presets • Not Used • Run Monitoring Only • Run Monitoring and Storage
Workflow	Presets • GenerateFASTQ
Workflow Type	Presets • No Index • Single Index • Dual Index • Custom
Index Read 1	Presets • 0 • 6 • 8 • Type a value between 0 and 20
Index Read 2	Presets • 0 • 6 • 8 • Type a value between 0 and 20
Paired End	Presets • True • False

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Field	Value
Read 1 Cycles	Presets • 251* • 151 • 101 • 51 • Type a value between 1 and 251 *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Read 2 Cycles	Presets • 251* • 151 • 101 • 51 • Type a value between 0 and 251 *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Use Custom Read 1 Primer	Select if applicable.
Use Custom Read 2 Primer	Select if applicable.
Use Custom Index Read 1 Primer	Select if applicable.
Use Custom Recipe	Select if applicable.
Custom Recipe Path	If you selected the Use Custom Recipe option, enter the path to the custom recipe file to be used.
Output Folder	Enter network path for sequencing run folder. For example: `\\networkshare\run_data`
Library Tube Barcode	Scan the library tube barcode.
Analysis Software Version	Enter the software version for BCL Convert Analysis for v2 sample sheet. Leave blank if not applicable.
Override Cycles	Enter the Override Cycles value for v2 sample sheet. Leave blank if not applicable.

5. Select Validate Run Setup and Generate Sample Sheet, and then select Next Steps to trigger the automation script. The script performs the following actions:
   - Validates the parameters entered on the Record Details screen.

- Generates the sample sheet and attaches it to the placeholder in the Files area on the Record Details screen.

> The v2 sample sheet generated does not include Cloud and Analysis sections. Contact Illumina Support for information on generating v2 sample sheets with these sections.

6. Select Next Steps.
   On the Assign Next Steps screen, the Next Step field for samples is prepopulated with Mark protocol as complete.
7. Select Finish Step.
   At this point in the workflow, the user interaction ends. The flow cell is queued for the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1) step.

Proceed to AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1).

> Do not add samples to the Ice Bucket or start the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1) step. The integration adds samples automatically.

Protocol 4: AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1)

This protocol contains a single fully automated step: AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1).

The integration starts the step automatically and data from the run is parsed back into Clarity LIMS. User interaction is not required, but you can review the stages of the step in Clarity LIMS (refer to Review Run Data).

Automations

The NovaSeqDx Run (NovaSeqDx v1.1) step contains two automations:

- Set Next Steps—This automation is disabled (Set to Not Used). By default, the Sequencer API is used to set the next step for output samples.
  The Illumina Preset Protocols (IPP) Package include a Set Next Steps automation configured on the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1) step. You can use this automation to override the default next step behavior. For more information, refer to *NovaSeq 6000Dx Integration v1.1 Configuration and User Interaction* on page 90.
- Update Lane Number—This automation is triggered automatically upon entry to the Record Details screen. To make sure that the Lane Number displays correctly, do not disable or modify this automation.

Review Run Data

Read summary metrics are recorded for the library pool. After the run is complete, open the step and review these metrics displayed on the Next Steps screen in the Step Data section and the Sample Details table.

Step Data Section

The following values populate the master step fields:

- Run ID
- Run Status
- Output Folder
- Current Cycle
- Current Read
- Loading Workflow Type
- Flow Cell ID
- Flow Cell Side
- Flow Cell Mode
- Flow Cell Part Number
- Flow Cell Lot Number
- Flow Cell Expiration Date
- Instrument ID
- Instrument Type
- Instrument Control Software Version
- Firmware Version
- RTA Version
- Sequencing Log

Sample Details Table

Summary metrics populate the global custom fields listed. Values are aggregated across all lanes. Some values (eg, Yield PF (Gb) R1) are summed, while others are averaged.

- Yield PF (Gb) R1
- Yield PF (Gb) R2
- Reads PF (M) R1
- Reads PF (M) R2
- Cluster Density (K/mm^2) R1
- Cluster Density (K/mm^2) R2
- %PF R1
- %PF R2

- % Bases >=Q30 R1
- % Bases >=Q30 R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- % Aligned R1
- % Aligned R2
- % Error Rate R1
- % Error Rate R2

How the Integration Works

The following steps summarize how the Sequencer API integration works. For details on sample sheet generation and the sequence of events involved in the NovaSeq run, refer to *NovaSeq 6000Dx Integration v1.1 Configuration and User Interaction* on page 90.

1. When setting up a run on the NovaSeq 6000Dx instrument, NVOS sends a request for the run recipe.
   a. The Sequencer API validates that samples and containers are correctly queued for the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1) step.
   b. Clarity LIMS sends a JSON response to NVOS. The response includes the run recipe information and a link to download the sample sheet (used with analysis software such as bcl2fastq2).
2. When the run starts, NVOS sends a RunStarted run status request. The Sequencer API then does the following:
   a. Validates reagent kit information in the RunStarted request:
      - Verifies that the reagent kit exists. If the reagent kit does not exist, the Sequencer API creates the kit and enables on the master step.
      - Verifies that the reagent kit is activated. If the reagent kit is not active, the Sequencer API activates it.
   b. Initiates the step for the queued samples. The step produces one output file placeholder per lane of the flow cell in use, based on the flow cell type.
   c. Records all relevant information from the RunStarted request on the step (such as reagent lots and step fields).
3. At the end of the run, NVOS sends one of the following run status requests:

- RunCompletedSuccessfully
- RunEndedByUSer
- RunErroredOut

The Sequencer API updates the step with any new information, which includes only the status and cycle/read information.

4. After primary analysis completes, NVOS sends a request containing the parsed run metrics. NVOS sends a request only when the run completes successfully. However, the API does not make this assumption and accepts the request regardless of status. The Sequencer API then does the following:
   a. Records the metrics into the fields on file placeholder outputs in Clarity LIMS.
   b. Completes the step in Clarity LIMS if the status is RunCompletedSuccessfully. For all other status options, the step remains in progress. This action is the default autocomplete step behavior. For more information, refer to *NovaSeq 6000Dx Integration v1.1 Configuration and User Interaction* on page 90.

   > *NovaSeq 6000Dx Integration v1.1 Configuration and User Interaction* on page 90 provides detailed information on how the following components work with the Sequencer API integration:
   >
   > - Sample sheet generation and contents
   > - NovaSeq 6000Dx run
   > - User authentication and login
   > - Run recipe contents

Troubleshooting

If an automation trigger does not appear to run its corresponding scripts, refer to the following sections in the Clarity LIMS API documentation:

- *Troubleshooting Automated Worker*
- *Troubleshooting Automation* in the API

If an error occurs that does not provide direction on how to proceed, (eg, the error is related to the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1) step) review the log file information. For more details, refer to the *Logging* on page 168 section.

If the automated run step starts, but does not complete, do as follows.

1. Use one of the following methods to open the in progress step in Clarity LIMS:
   - Log in to the default user account.
   - In Lab View, find the step in the Recent Activities pane.

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- Search for the step in Clarity LIMS. You can use the Library Tube or Flowcell barcode as the search term. The steps depend on whether the search is a NovaSeqDx Standard or NovaSeqDx Xp run.

2. On the Record Details screen, the Sequencing Log multiline text field contains logging information. If unable to reach the Record Details screen, or if the Sequencing Log field does not contain enough information to resolve the issue, review the `sequencer-api.log` file. For details, refer to *Logging on page 168*.
3. Contact the Clarity LIMS support team. Provide the relevant information from the troubleshooting steps already performed.

Logging

In addition to updating the Sequencing Log multiline text field on the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1) step, the Sequencer API writes a detailed log file to `/opt/gls/clarity/tomcat/current/logs/sequencer-api.log`.

Log messages include the Library Tube ID and Flow Cell ID whenever the messages are related to a sequencing run-related request. In the Clarity LIMS workflows, these IDs are recorded as container names.

Some log messages are not directly related to a sequencing run request, such as downloading the sample sheet. In this case, the file LIMS ID and file name are included in log messages. The sample sheet downloaded by the sequencing system has a name that includes the sequencing container ID (Library Tube for Standard or Flowcell for Xp). The relevant container name search finds most of the log messages for this request. However, the LIMS ID of the file must be known in order to retrieve all messages related to the file download. The LIMS ID is found in the Clarity LIMS API and, for sample sheets used in a run, in the recipe response (which can also be found in the log file).

Example lines from the log file:

```
2021-06-19 21:32:19.705 INFO --- [] SequencerAPIApplication : Started
SequencerAPIApplication in 7.026 seconds (JVM running for 15.964)
...
2021-06-19 21:34:44.564 INFO --- [Library Tube Id=NV1234567-LIB, Flow Cell
Id=RM123DSXX] LimsApiLookupUtil : Searching for containers with name 'NV1234567-
LIB' or 'RM123DSXX'
....
2021-06-19 21:35:27.158 INFO --- [] FileService : Successfully retrieved file
'NV1234567-LIB.csv' with LIMS ID 40-51 through the API.
```

NovaSeq 6000Dx Integration v1.1 Installation

The Illumina NovaSeq 6000Dx Integration v1.1 supports the integration between Clarity LIMS (v5.1 or later) and the NovaSeq 6000Dx instrument. The integration includes the following changes:

- Updates to the NovaSeq 6000Dx library tube and flow cell barcodes that are accepted by the workflow.
- Fixes to the Denial of Service (CVE-2022-22970) and Integer Overflow (CVE-2022-22976) vulnerabilities.

This document provides instructions for installing NovaSeq 6000Dx Integration v1.1 and describes the components that are installed in the default configuration.

Integration Overview

Illumina NovaSeq 6000Dx Integration v1.1 includes the following features and functionality:

- Preconfigured NovaSeqDx v1.1 workflow that maps to lab protocols and instrument runs.
- The following preconfigured protocols:
  - Run Format (NovaSeqDx v1.1)
  - NovaSeqDx Standard (NovaSeqDx v1.1) (supports the loading of pooled libraries into a library tube and the IVD sequencing run for the S2 and S4 flow cells)
  - NovaSeqDx Xp (NovaSeqDx v1.1) (supports individual lane loading on the NovaSeq 6000Dx)
  - AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1)
- Automated generation of the v2 sample sheet. This file is automatically uploaded to the sequencing instrument through the Sequencer API and is used to set up the run.
- Automated validation of run setup information. This information is uploaded to the NovaSeq 6000Dx Operating Software (NVOS) through the Sequencer API. It is used to create the run recipe and initiate the run.
- Automated tracking of the following NovaSeq 6000Dx sequencing run information, and parsing of run statistics into Clarity LIMS:
  - Progress and metrics of sequencing run
  - Per-instrument sequencing runs (tracked as part of the Run ID field value)
  - Sequencing run parameters
  - Sequencing run data directory location
  - Real-Time Analysis v3 (RTA3) version and other run-specific information
- [Optional] Preconfigured Library Prep Validation v2.3.0 workflow used for validation purposes. This workflow contains a single-step protocol that models the library prep required to produce normalized libraries that are ready for the NovaSeqDx v1.0 workflow. For more information, refer to *NovaSeq 6000Dx Integration v1.1 Validation and Troubleshooting* on page 148.

Prerequisites

Prerequisite 1: Illumina Preset Protocols (IPP) v2.5

The Illumina NovaSeq 6000Dx Integration Package v1.1 depends on the configuration provided in Illumina Protocols (IPP) v2.5.

The installation procedure provided in this document includes steps that install the following IPP configurations on the server used for the NovaSeq 6000Dx integration installation:

- QC Protocols Base configuration
- NovaSeqDx configuration
- Library Prep Validation configuration (optional, but recommended)

For details on IPP v2.5 installation and configuration, refer to the *Illumina Preset Protocols* documentation.

Prerequisite 2: Proxy Update (Illumina Cloud Hosted Installation Only)

The Illumina cloud hosted installation configures an additional include directive for `/etc/httpd/conf.d/clarity.conf` in the proxy configuration. Additional proxy configuration files for the Sequencer API service are expected to be placed in the `/etc/httpd/conf.d/clarity` folder.

Prerequisite 3: Secret Utility Configuration

If Clarity LIMS Secret Utility is not installed or configured, use the following information to configure Secret Utility:

Installation on a hosted system with Clarity LIMS (v5.4 or later)

The Clarity LIMS installation tooling configures this installation. No additional configuration is necessary.

Installation on Clarity LIMS (v5.3 or later)

The installation includes the following components:

- Tenant DB Password
- Clarity DB Password
- FTP password
- LDAP manager password (if LDAP is enabled)
- LabLink DB password (for Clarity LIMS v5.3 LabLink only)
- Reporting DB password (if installed)
- RabbitMQ password

For more information on Secret Utility configuration, refer to the *Clarity LIMS Core Functionality* documentation.

Sequencer API RPM Installation Components

NovaSeq 6000Dx Integration v1.1 is distributed as the BaseSpaceLIMS-sequencer-api RPM package. This package must be installed on the Clarity LIMS server.

The BaseSpaceLIMS-sequencer-api RPM installs the following files and scripts:

- Sequencer API WAR file
- `application.yml` configuration file
- `configure_sequencer_api_proxy.sh` configuration script
- `configure_sequencer_api_env.sh` configuration script
- `configure_sequencer_api_application.sh` configuration script

Sequencer API WAR

The RPM installs the Sequencer API WAR file into the same Tomcat container as Clarity LIMS. The WAR file is installed to the following location:
`/opt/gls/clarity/tomcat/current/webapps/Illumina#Sequencer#v2##2.5.0.0.war`

The version number can differ.

The WAR file is deployed automatically when Clarity LIMS launches. Because the RPM installs a new WAR file, Clarity LIMS is automatically stopped during installation of the RPM. Restart Clarity LIMS before installing the NovaSeq and Library Prep Validation workflow configuration from the IPP.

Configuration File

The `application.yml` configuration file is installed to the following location:

`/opt/gls/clarity/extensions/sequencer-api/application.yml`

The file properties are configured with a script during the installation process.

For a complete list of all properties included in the `application.yml` file, refer to *NovaSeq 6000Dx Integration v1.1 Configuration and User Interaction* on page 90.

Configuration Scripts

The scripts in the following table are installed to the following location:

`/opt/gls/clarity/config`

During installation, the RPM runs these scripts as they are needed.

Script Name	User	Description
configure_sequencer_api_proxy.sh	root	Adds configuration to the proxy to allow communication with the Sequencer API after Tomcat has been started. Run this script in the following scenarios: • When the Sequencer API is first installed. • When reconfiguring the Clarity LIMS proxy. This process happens until a version of Clarity LIMS that includes the necessary configuration files from `/etc/httpd/clarity/*.conf` is available. Not required for Illumina cloud hosted installations.
configure_sequencer_api_env.sh	root	Adds Secret Management setting into the Clarity LIMS Tomcat configuration. Run this script when the Sequencer API is first installed on a Secret Util-enabled Clarity LIMS.

For Research Use Only. Not for use in diagnostic procedures.

Script Name	User	Description
configure_sequencer_api_application.sh	glsjboss	• Configures the installed `application.yml` file. • Sets the Clarity LIMS URL and the key to use when signing access tokens. Run this script when the Sequencer API is first installed. **!** If you run the script multiple times, the script reconfigures each setting to its default. The only exception is the signing key. For this setting, you are asked if you want a reset. If the reset is done, all previously issued tokens are no longer valid.

Installation Steps

NovaSeq 6000Dx Integration v1.1 supports RedHat Enterprise Linux and CentOS up to v7.9. Use v7.9 due to mitigate security risks. CentOS v6 is not supported.

If running the sequencing service and Automation Worker on the same instance, the instance must also be running a supported version of the Linux operating system.

[Optional] Install QC Protocols

If you are using PhiX control, install the base configuration of the QC Protocols from IPP as follows.

1. Log in as the root user on the server being used to install the integration.
2. Use the following yum command to install the IPP RPM:

```
yum install BaseSpaceLIMS-Illumina-Preset-Protocols --enablerepo=<< repo name info from support >>
```

   The `--enablerepo` command-line argument must be included to enable the repo. The Illumina Support team can provide you with the repo file with the appropriate naming convention.
3. Enter `y` to confirm that you want to proceed with the RPM and package installation.
4. Make sure that NGS Extension Package v5.22 is installed.
   If this package is not installed, you must install it or upgrade the existing package.
5. As the glsjboss user, install the `QC_Protocols.qc-protocols` configuration from the following location:

```
/opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o install QC_
Protocols.qc-protocols.
```

For information on IPP installer commands, parameters, and options, refer to *Illumina Preset Protocols Integration v2.5 Installation and Configuration* in the *Illumina Preset Protocols* documentation.

Install Sequencer API

Install the Sequencer API as follows.

1. On the Clarity LIMS server, log in as the root user.
2. Use the following yum command to install the RPM:

```
yum install BaseSpaceLIMS-sequencer-api --enablerepo=< repo name info from
support >
```

   The `--enablerepo` command-line argument must be included to enable the repository. The Illumina Support team can provide you with the repo file with the appropriate naming convention.

3. Enter `y` to confirm that you want to proceed with the RPM installation.
4. If necessary, install the Secret Utility Package.
   For more information on this package, refer to *Prerequisite 3: Secret Utility Configuration* on page 170.
5. As the glsjboss user, run `configure_sequencer_api_application.sh` from the following location:

```
bash /opt/gls/clarity/config/configure_sequencer_api_application.sh
```

6. Enter values for the presented as follows.
   You can also press Enter to accept the default values in the `application.yml` configuration file. For more information about the `application.yml` properties, refer to *NovaSeq 6000Dx Integration v1.1 Configuration and User Interaction* on page 90.
   - Would you like to use the apiuser user for communication with Clarity? [Y]
   - Enter required value for Token Expiry (hours) [88]
7. Update the Clarity LIMS proxy for the Sequencer API as follows.
   - For an on-premise system, run the following command:

```
bash /opt/gls/clarity/config/configure_sequencer_api_proxy.sh
```

   - For an Illumina cloud hosted system, make sure that the proxy has been updated as follows. Place the `sequencer-api.include` file in `/etc/httpd/conf.d/clarity`.

```
<Location "/Illumina/Sequencer/v2">
ProxyPass ajp://localhost:9009/Illumina/Sequencer/v2
ProxyPassReverse ajp://localhost:9009/Illumina/Sequencer/v2
</Location>
```

8. Update the Clarity LIMS Tomcat configuration as follows.

a. Make sure that Secret Utility is installed and configured. For more information, refer to *Prerequisite 3: Secret Utility Configuration* on page 170.

b. Run the following command to add the Secret Utility configuration to Clarity LIMS:

```
bash /opt/gls/clarity/config/configure_sequencer_api_env.sh
```

> This script modifies `/opt/gls/clarity/tomcat/current/bin/setenv.sh` for Clarity LIMS Tomcat to load the Secret Utility environment variable file (`/etc/profile.d/claritysecret.sh`) during startup.

c. Use the following command to Start Clarity LIMS:

```
/opt/gls/clarity/bin/run_clarity.sh start
```

Install NovaSeqDx and Library Prep Validation Workflow Configuration

Install the NovaSeqDx and Library Prep workflow configuration from IPP as follows.

1. As the glsjboss user, run the following command to install the NovaSeq 6000Dx v1.1 workflow configuration:

```
bash /opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o install
Illumina_Instruments.novaseqdx-v1.1
```

> The installer validates the import of the workflow and provides the Warning/Error details in STDOUT, which allows you to either proceed with the import or cancel it.

2. [Optional] Run the following command to install the Library Prep Validation v2.3.0 workflow:

```
/opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o install
Illumina_Instruments.Library-Prep-Validation-v2.3
```

This step is optional, but recommended. The Library Prep Validation v2.3.0 workflow allows you to validate the NovaSeqDx v1.1 workflow.

Check Sequencer API

Make sure that the Sequencer API is working as follows.

1. Visit `https://<< Clarity LIMS URL >>/Illumina/Sequencer/v2/sequencing-run/login`. This page returns the following URL string:

```
https://<< Clarity LIMS URL >>/Illumina/Sequencer/v2/oauth/authorize?client_
id=illumina_sequencer&amp;redirect_uri=../sequencer_login&amp;response_
type=token
```

2. Copy and paste the URL string into the browser. The Clarity LIMS Sign In page opens.
3. Enter sign in credentials.
4. Select Sign In. A Successfully Authenticated message displays.

[Optional] Configure `application.yml` Properties

The installer script configures the `application.yml` configuration properties by prompting for values. You can also configure these properties by editing the `application.yml` file, and then restarting Clarity LIMS. Configuring these properties is optional.

For more information, refer to *NovaSeq 6000Dx Integration v1.1 Configuration and User Interaction* on page 90.

Workflows, Protocols, and Steps Installed

NovaSeq 6000Dx Integration v1.1 includes the workflows, protocols, and steps listed in the following table.

Package	Workflow	Protocols	Steps
IPP v2.5	NovaSeqDx v1.1	Run Format (NovaSeqDx v1.1)	1. Define Run Format (NovaSeqDx v1.1)
		NovaSeqDx Standard (NovaSeqDx v1.1)	1. Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.1) 2. Dilute and Denature (NovaSeqDx v1.1)
		NovaSeqDx Xp (NovaSeqDx v1.1)	1. Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.1) 2. Dilute, Denature, and ExAmp (NovaSeqDx v1.1) 3. Load to Flowcell (NovaSeqDx v1.1)
		AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1)	1. AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1)
IPP v2.5	Library Prep Validation v2.3.0	Library Prep Validation v2.3.0	1. Library Prep Validation v2.3.0

For detailed descriptions of the steps and automations included in each protocol, and details on other components in the configuration, refer to *NovaSeq 6000Dx Integration v1.1 Configuration and User Interaction* on page 90.

For instructions on using the Library Prep Validation v2.3.0 protocol, refer to *NovaSeq 6000Dx Integration v1.1 Validation and Troubleshooting* on page 148.

Instrument Software

The NovaSeq 6000Dx instrument software includes the following components:

- NovaSeq 6000Dx Operating Software (NVOS)—Contains the user interface for setting up the sequencing run. Responsible for controlling the instrument and acquiring the images.
- Real-Time Analysis v3 (RTA3)—Takes the images generated by the first module, processes, and analyzes them. Makes sure that data files are created and copied to the final destination folder.
- Sequencing Analysis Viewer (SAV)—Displays the important quality metrics generated by the RTA3 software.
- NovaSeq 6000Dx recipes—Provides system operation instructions for use with NovaSeq 6000Dx reagent kits for SP, S1, S2, and S4 flow cells.
- Universal Copy Service—Copies output files to destinations such as final destination folder and/or BaseSpace Sequence Hub (when the instrument is configured for use with BaseSpace Sequence Hub).

Instrument Integration Configuration

For the NovaSeq 6000Dx API integration to work, the instrument must be able to communicate with Clarity LIMS through the API. Complete the following steps to configure the NVOS and confirm that you can access Clarity LIMS from the instrument.

1. Launch NVOS and wait for the initialization process to complete.
2. On the NVOS Instrument Settings page, do as follows.
   a. Select the Global Settings tab.
   b. Under Instrument Mode, select one of the following modes:
      - Allow users to switch between IVD and RUO
      - IVD Only
      - RUO Only
3. If you selected IVD Only instrument mode, select the IVD Settings tab and complete the following steps:
   a. Configure Run Setup as follows.
      - Select the Select runs from the LIMS server option.
      - Enter the Clarity LIMS URL (eg, https://yourserver.claritylims.com).
   b. [Optional] Under Output Location, enter the output folder location. You can also specify the output folder location when setting up a run in NVOS.

4. If you selected RUO Only instrument mode, select the RUO Settings tab and complete the following steps:
   a. Configure Run Setup as follows.
      - Select the Select runs from the LIMS server option.
      - Enter the Clarity LIMS URL(eg, https://yourserver.claritylims.com).
   b. Configure Default Work Type as follows.
      - [Optional] If selection support between NovaSeq Xp or NovaSeq Standard is required for the workflow, select the Enable workflow selection checkbox.
      - Select the required default workflow type: NovaSeq Standard or NovaSeq Xp.
   c. [Optional] Configure Output Location as follows.
      - [Optional] Enter the output folder location. You can also specify the output folder location when setting up a run in NVOS.
      - [Optional] To upload and store run data in the cloud server, select the BaseSpace Sequence Hub checkbox. Select a Configuration option (Run Monitoring and Storage or Run Monitoring Only) and select your Hosting Location from the drop-down list.
5. Select Save to complete the configuration.
6. On the NVOS home page, you can now select Sequence to log into Clarity LIMS and start a run.

NovaSeq 6000Dx Integration v1.1 Manual Upgrade

NovaSeq 6000Dx Integration Package v1.1 includes the NovaSeqDx Sequencing v1.1 workflow that is required for the integration to work.

If you do not want to upgrade your existing NovaSeqDx Sequencing workflow to the latest workflow in IPP, update the workflow configuration manually.

Update Calculate Volumes Automation on Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.0) Step

Update the Calculate Volumes automation on the Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.0) step as follows.

1. From Configuration, select the Automation tab.
2. Under the Step Automation tab, search for the Calculate Volumes automation for the NovaSeqDx Standard (NovaSeqDx v1.0) step.
3. Change values in the existing command line as follows.
   Changes to the existing command line are in red.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} -t true -log {compoundOutputFileLuid1} \
```

```
script:evaluateDynamicExpression \
-h false \
-exp 'step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: +
1' \
script:evaluateDynamicExpression \
-h false \
-exp 'step.::PhiX Volume (ul):: = !step.hasValue(::% PhiX (2.5nM) Spike-In::) ?
0 : step.::% PhiX (2.5nM) Spike-In::; if (input.::Flowcell Type:: == ::SP::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 100;
step.::PhiX Volume (ul):: *= 0.6 }; if (input.::Flowcell Type:: == ::S1::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 100;
step.::PhiX Volume (ul):: *= 0.6 }; if (input.::Flowcell Type:: == ::S2::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 150;
step.::PhiX Volume (ul):: *= 0.9 }; if (input.::Flowcell Type:: == ::S4::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 310;
step.::PhiX Volume (ul):: *= 1.9 }; if (step.::PhiX Volume (ul):: == 0) {
step.::PhiX Volume (ul):: = :::: }; input.::Per Sample Volume (ul):: =
(((input.::Final Loading Concentration (pM):: * 5 / 1000) / input.::Normalized
Molarity (nM)::) * step.::Bulk Pool Volume (ul)::) / step.::Number of Samples in
Pool::' \
script:calculate_adjusted_per_sample_volume \
script:evaluateDynamicExpression \
-h false \
-exp 'step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: +
input.::Adjusted Per Sample Volume (ul)::' \
script:evaluateDynamicExpression \
-h false \
-exp 'if (step.::Total Sample Volume (ul):: >= step.::Bulk Pool Volume (ul)::)
{output.::RSB Volume (ul):: = 0} else {output.::RSB Volume (ul):: = step.::Bulk
Pool Volume (ul):: - step.::Total Sample Volume (ul)::} ; output.::Flowcell
Type:: = input.::Flowcell Type:: ; output.::Loading Workflow Type:: =
input.::Loading Workflow Type:: ; if ( input.::Flowcell Type:: == ::SP:: ) {
output.::Volume of Pool to Denature (ul):: = 100 ; output.::NaOH Volume (ul):: =
25 ; output.::Tris-HCl Volume (ul):: = 25 } ; if ( input.::Flowcell Type:: ==
::S1:: ) { output.::Volume of Pool to Denature (ul):: = 100 ; output.::NaOH
Volume (ul):: = 25 ; output.::Tris-HCl Volume (ul):: = 25 } ; if (
input.::Flowcell Type:: == ::S2:: ) {output.::Volume of Pool to Denature (ul)::
= 150 ; output.::NaOH Volume (ul):: = 37 ; output.::Tris-HCl Volume (ul):: = 38
} ; ; if ( input.::Flowcell Type:: == ::S4:: ) { output.::Volume of Pool to
Denature (ul):: = 310 ; output.::NaOH Volume (ul):: = 77 ; output.::Tris-HCl
Volume (ul):: = 78 }' \
```


For Research Use Only. Not for use in diagnostic procedures.

```
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Standard_Bulk_
Pool1.csv \
-o 1.csv \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Standard_Bulk_
Pool2.csv \
-o 2.csv \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Standard_Bulk_
Pool3.csv \
-o 3.csv \
&& cat 1.csv 2.csv 3.csv > {compoundOutputFileLuid0}.csv \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} -t
true -log {compoundOutputFileLuid1} \
script:evaluateDynamicExpression \
-h false \
-exp 'step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: -
1; step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: -
input.::Adjusted Per Sample Volume (ul)::'"
```

4. Select Save.

Update Validate Library Tube Barcode Automation on Dilute and Denature (NovaSeqDx v1.0) Step

Update the Validate Library Tube Barcode automation on the Dilute and Denature (NovaSeqDx v1.0) step as follows.

1. From Configuration, select the Automation tab.
2. Under the Step Automation tab, search for the Validate Library Tube Barcode automation for the Dilute and Denature (NovaSeqDx v1.0) step.
3. Update the existing command line as follows.
   Changes to the existing command line are in red.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
script:evaluateDynamicExpression -exp 'if ((input.::NovaSeqDx Run Mode:: ==
::DX:: && !output.container.name.matches(::^DX[0-9]{7}-[A-Z]{3}$::)) ||
```

```
(input.::NovaSeqDx Run Mode:: == ::RUO:: && !output.container.name.matches(::[A-Z]{2}[0-9]{7}-[A-Z]{3}::))) { fail(::Invalid Library Tube Barcode. Please verify and try again.::) }; output.::Flowcell Type:: = input.::Flowcell Type::; output.::Loading Workflow Type:: = input.::Loading Workflow Type::' -log {compoundOutputFileLuid1}"
```

4. Select Save.

Update Calculate Volumes Automation on Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.0) Step

Update the Calculate Volumes automation on the Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.0) step as follows.

1. From Configuration, select the Automation tab.
2. Under the Step Automation tab, search for the Calculate Volumes automation for the NovaSeqDx Xp (NovaSeqDx v1.0) step.
3. Update the existing command line as follows.
   Changes to the existing command line are in red.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} -t true -log {compoundOutputFileLuid1} \
script:evaluateDynamicExpression \
-h false \
-exp 'step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: + 1' \
script:evaluateDynamicExpression \
-h false \
-exp 'step.::PhiX Volume (ul):: = !step.hasValue(::% PhiX (0.25nM) Spike-In::) ? 0 : step.::% PhiX (0.25nM) Spike-In::; if (input.::Flowcell Type:: == ::SP::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Lanes to Sequence:: * 18;
step.::PhiX Volume (ul):: *= 0.7 }; if (input.::Flowcell Type:: == ::S1::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Lanes to Sequence:: * 18;
step.::PhiX Volume (ul):: *= 0.7 }; if (input.::Flowcell Type:: == ::S2::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Lanes to Sequence:: * 22;
step.::PhiX Volume (ul):: *= 0.8 }; if (input.::Flowcell Type:: == ::S4::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Lanes to Sequence:: * 30;
step.::PhiX Volume (ul):: *= 1.1 }; if (step.::PhiX Volume (ul):: == 0) {
step.::PhiX Volume (ul):: = :::: }; input.::Per Sample Volume (ul):: = (((input.::Final Loading Concentration (pM):: * 5 / 1000) / input.::Normalized Molarity (nM)::) * step.::Bulk Pool Volume (ul)::) / step.::Number of Samples in Pool::' \
```

```
script:calculate_adjusted_per_sample_volume \
script:evaluateDynamicExpression \
-h false \
-exp 'step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: +
input.::Adjusted Per Sample Volume (ul)::' \
script:evaluateDynamicExpression \
-h false \
-exp 'if (step.::Total Sample Volume (ul):: >= step.::Bulk Pool Volume (ul)::)
{output.::RSB Volume (ul):: = 0} else {output.::RSB Volume (ul):: = step.::Bulk
Pool Volume (ul):: - step.::Total Sample Volume (ul)::} ; output.::Flowcell
Type:: = input.::Flowcell Type:: ; output.::Loading Workflow Type:: =
input.::Loading Workflow Type::' \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_Bulk_Pool.csv
\
-o {compoundOutputFileLuid0}.csv \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_Bulk_
Pool2.csv \
-o append.csv \
&& cat append.csv >> {compoundOutputFileLuid0}.csv \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} -t
true -log {compoundOutputFileLuid1} \
script:evaluateDynamicExpression \
-h false \
-exp 'step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: -
1; step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: -
input.::Adjusted Per Sample Volume (ul)::'"
```

4. Select Save.

Update Calculate Volumes Automation on Dilute, Denature & ExAmp (NovaSeqDx v1.0) Step

Update the Calculate Volumes automation on the Dilute, Denature & ExAmp (NovaSeqDx v1.0) step as follows.

1. From Configuration, select the Automation tab.

2. Under the Step Automation tab, search for the Calculate Volumes automation for the Dilute, Denature & ExAmp (NovaSeqDx v1.0) step.
3. Update the existing command line as follows.
   Changes to the existing command line are in red.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-t true \
-h false \
-exp 'if ( input.::Flowcell Type:: == ::SP:: ) { output.::BP Aliquot Volume
(ul):: = 18 ; output.::NaOH Volume (ul):: = 4 ; output.::Tris-HCl Volume (ul)::
= 5 ; step.::DPX1 Volume (ul):: = 126 ; step.::DPX2 Volume (ul):: = 18 ;
step.::DPX3 Volume (ul):: = 66 ; output.::Mastermix per Lane (ul):: = 63} ;if (
input.::Flowcell Type:: == ::S1:: ) { output.::BP Aliquot Volume (ul):: = 18 ;
output.::NaOH Volume (ul):: = 4 ; output.::Tris-HCl Volume (ul):: = 5 ;
step.::DPX1 Volume (ul):: = 126 ; step.::DPX2 Volume (ul):: = 18 ; step.::DPX3
Volume (ul):: = 66 ; output.::Mastermix per Lane (ul):: = 63} ; if (
input.::Flowcell Type:: == ::S2:: ) { output.::BP Aliquot Volume (ul):: = 22 ;
output.::NaOH Volume (ul):: = 5 ; output.::Tris-HCl Volume (ul):: = 6 ;
step.::DPX1 Volume (ul):: = 126 ; step.::DPX2 Volume (ul):: = 18 ; step.::DPX3
Volume (ul):: = 66 ; output.::Mastermix per Lane (ul):: = 77} ; if (
input.::Flowcell Type:: == ::S4:: ) { output.::BP Aliquot Volume (ul):: = 30 ;
output.::NaOH Volume (ul):: = 7 ; output.::Tris-HCl Volume (ul):: = 8 ;
step.::DPX1 Volume (ul):: = 315 ; step.::DPX2 Volume (ul):: = 45 ; step.::DPX3
Volume (ul):: = 165 ; output.::Mastermix per Lane (ul):: = 105} ;
output.::Flowcell Type:: = input.::Flowcell Type:: ; output.::Loading Workflow
Type:: = input.::Loading Workflow Type::' \
-log {compoundOutputFileLuid1} \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {processURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Xp_Working_Pool.csv \
-o {compoundOutputFileLuid0}.csv \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Xp_Working_Pool2.csv \
-o append.csv \
&& cat append.csv >> {compoundOutputFileLuid0}.csv"
```

4. Select Save.

Update Validate Flowcell Barcode Automation on Load to Flowcell (NovaSeqDx v1.0) Step

Update the Validate Flowcell Barcode automation on the Load to Flowcell (NovaSeqDx v1.0) step as follows.

1. From Configuration, select the Automation tab.
2. Under the Step Automation tab, search for the Calculate Volumes automation for the Load to Flowcell (NovaSeqDx v1.0) step.
3. Update the existing command line as follows.
   Changes to the existing command line are in red.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-exp 'if (input.::Flowcell Type:: == ::SP:: && !output.container.name.matches(
::[A-Z0-9]{5}DR[A-Z0-9]{2}:: ) ) {fail ( ::Invalid Flowcell Barcode. Please
verify and try again.:: ) } ; if (input.::Flowcell Type:: == ::S1:: &&
!output.container.name.matches( ::[A-Z0-9]{5}DR[A-Z0-9]{2}:: ) ) {fail (
::Invalid Flowcell Barcode. Please verify and try again.:: ) } ; if
(input.::Flowcell Type:: == ::S2:: && !output.container.name.matches( ::[A-Z0-9]
{5}DM[A-Z0-9]{2}:: ) ) {fail ( ::Invalid Flowcell Barcode. Please verify and try
again.:: ) } ; if (input.::Flowcell Type:: == ::S4:: &&
!output.container.name.matches( ::[A-Z0-9]{5}DS[A-Z0-9]{2}:: ) ) {fail (
::Invalid Flowcell Barcode. Please verify and try again.:: ) } ;
output.::Flowcell Type:: = input.::Flowcell Type:: ; output.::Loading Workflow
Type:: = input.::Loading Workflow Type::' \
-log {compoundOutputFileLuid1}"
```

4. Select Save.

NovaSeq 6000Dx Integration v1.1 Release Notes

These Release Notes describe the key changes to software components for the Clarity LIMS NovaSeqDx Integration Package version 1.1.

Compatibility

This version of the integration package is compatible with the following software:

- BaseSpace Clarity LIMS v5.1 and later
- NGS Extensions Package v5.22 and later
- Illumina Preset Protocols (IPP) v2.5.0 and later
- NovaSeq 6000Dx Operating Software (NVOS) v2.3.1

New Features

Features provided with this release of the integration package are as follows.

- Updates the Validate Flowcell Barcode automation (in the Load to Flowcell step of the NovaSeq Xp protocol) to support future flow cell barcode formats.
- Fixes Denial of Service (CVE-2022-22970) and Integer Overflow (CVE-2022-22976) vulnerabilities.

Defects Fixed

The following Calculate Volumes automation script values are now in sync with the *NovaSeq 6000 Denature and Dilute Libraries Guide*:

- PhiX
- NaOH
- Tris-HCl
- Bulk pool volume

The Validate Library Tube Barcode automation (in the Dilute and Denature step of the NovaSeqDx Standard protocol for Dx run mode) correctly validates Dx library tubes.

Known Issues

None

NovaSeq 6000Dx Integration v1.0 Configuration and User Interaction

The Illumina NovaSeq 6000Dx Integration Package v1.0 supports the integration of Clarity LIMS to Illumina NovaSeq 6000Dx instruments. This integration supports Clarity LIMS v5.1 and later.

For instructions on validating and troubleshooting the Illumina NovaSeq 6000Dx Integration, refer to *NovaSeq 6000Dx Integration v1.0 Validation and Troubleshooting* on page 244.

The configuration provided in this integration has been established to support NovaSeq 6000Dx lab processes. Any configuration changes to protocols or workflows—including renaming protocols, steps, and fields—could break the process.

Overview

The integration includes the following features:

- Preconfigured NovaSeqDx v1.0 workflow that maps to lab protocols and instrument runs.
- The following preconfigured protocols:
  - Run Format (NovaSeqDx v1.0)
  - NovaSeqDx Standard (NovaSeqDx v1.0) (supports the loading of pooled libraries into a library tube)

  - NovaSeqDx Xp (NovaSeqDx v1.0) (supports individual lane loading on the NovaSeq)
  - AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.0)
- Automated generation of v2 sample sheet. This file is automatically uploaded to the instrument via the API.
- Automatic validation of run setup information. This information is used to create the run recipe and initiate the run.
- Automated tracking of the NovaSeq sequencing run and parsing of run statistics into Clarity LIMS, including the following features:
  - Progress and metrics of sequencing run
  - Per-instrument sequencing runs (tracked as part of the Run ID field value)
  - Sequencing run parameters
  - Sequencing run data directory location
  - Real-Time Analysis v3 (RTA3) version and other run-specific information
  - [Optional] Preconfigured Library Prep Validation v2.2.0 workflow used for validation purposes only. The workflow contains a single-step protocol that models the library prep required to produce normalized libraries that are ready for the NovaSeqDx v1.0 workflow. For more information, refer to *NovaSeq 6000Dx Integration v1.0 Validation and Troubleshooting* on page 244. The Library Prep Validation v2.2.0 workflow is available in the Illumina Preset Protocols (IPP) v2.4 package.

Prerequisites and Assumptions

Samples enter the NovaSeqDx v1.0 workflow as normalized libraries. It is assumed that the following steps have completed before samples are assigned to the workflow:

- Samples have been accessioned into the Clarity LIMS.
- Samples have been run through QC and library prep.
- Samples have been normalized, and the value is captured in a field called Normalized Molarity (nM).

For more information on sample accessioning, refer to *Sample Accessioning* and *Upload and Modify Samples* in the Getting Started section of the Clarity LIMS Core Functionality documentation.

You can assign samples to workflows automatically, using a routing script, or manually—from the Projects & Samples dashboard. Refer to *Assign and Process Samples* in the Clarity LIMS Core Functionality documentation.

Workflows, Protocols, and Steps

The Illumina NovaSeq 6000Dx Integration Package v1.0 includes the following workflows:

- NovaSeqDx v1.0
- Library Prep Validation v2.2.0 (optional, but recommended for validation purposes)

The following table describes the protocols and steps included in these workflows.

Table 80 NovaSeq 6000Dx Integration Package v1.0 Protocols and Steps

Protocol Name	Protocol Purpose	Protocol Steps
	Library Prep Validation v2.2.0 Workflow	
Library Prep Validation v2.2.0	Included for validation purposes only, this protocol models the library prep steps required to advance samples to the Run Format (NovaSeqDx v1.0) protocol. The protocol contains a single step - Library Prep Validation v2.2.0. After this step, a routing script sends the samples to the first step of the NovaSeqDx v1.0 workflow - Define Run Format (NovaSeqDx v1.0).	1. Library Prep Validation v2.2.0
	NovaSeqDx v1.0 Workflow	
Run Format (NovaSeqDx v1.0)	Allows for the assignment of per sample values for the following properties: • NovaSeqDx Run Mode: Select either RUO or DX. • Loading Workflow Type: Select either NovaSeq Standard or NovaSeq Xp. • Normalized Molarity (nM): Enter a value for each sample. • Flowcell Type: Select from options SP, S1, S2, or S4. • Final Loading Concentration (pM): Select from options 225 (PCR-free workflows) or 400 (Nano workflows), or enter a different value. Compares the Normalized Molarity value of each sample with the Minimum Molarity value. Routing script sends samples to the NovaSeqDx Standard or NovaSeqDx Xp protocol, according to the selected Loading Workflow Type. Samples with Normalized Molarity less than Minimum Molarity are removed from the workflow.	1. Define Run Format (NovaSeqDx v1.0)

Protocol Name	Protocol Purpose	Protocol Steps
NovaSeqDx Standard (NovaSeqDx v1.0)	Samples are pooled and added to the library tube in preparation for the NovaSeq 6000Dx run. The run setup information is validated and a sample sheet is generated. Routing script sends the library tube to the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.0) protocol.	1. Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.0) 2. Dilute and Denature (NovaSeqDx v1.0)
NovaSeqDx Xp (NovaSeqDx v1.0)	Samples are pooled and added to lanes on the NovaSeq 6000Dx flow cell. The option selected in the Define Run Format (NovaSeqDx v1.0) step determines the flow cell type. The run setup information is validated and a sample sheet is generated. Routing script sends flow cell to the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.0) protocol.	1. Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.0) 2. Dilute, Denature & ExAmp (NovaSeqDx v1.0) 3. Load to Flowcell (NovaSeqDx v1.0)
AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.0)	All samples complete the workflow by going through this protocol. This protocol contains one fully automated step. Do not add samples to the Ice Bucket or start the step. The integration starts the step automatically.	1. AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.0)

Validation Workflow

The Library Prep Validation v2.2.0 workflow allows for validation of the system after installation is complete. For details, refer to *NovaSeq 6000Dx Integration v1.0 Validation and Troubleshooting* on page 244.

Step Automations

The following table lists the step automations included in the NovaSeq 6000Dx v1.0 workflow. Automation details are provided in the documentation for each step.

For details on the validation workflow, refer to *NovaSeq 6000Dx Integration v1.0 Validation and Troubleshooting* on page 244.

Table 81 NovaSeq 6000Dx v1.0 Workflow Step Automations

Protocol	Step	Automation
1. Run Format (NovaSeqDx v1.0)	1. Define Run Format (NovaSeqDx v1.0)	Routing Script Set Next Steps
2. NovaSeqDx Standard (NovaSeqDx v1.0)	1. Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.0)	Calculate Volumes Set Next Step Validate Inputs Flowcell Type and Single Pool Validate Single NovaSeqDx Run Mode Validate Unique Indexes (Not used. The core Clarity LIMS configuration for pooling handles this functionality.)
	2. Dilute and Denature (NovaSeqDx v1.0)	Routing Script Validate Library Tube Barcode Validate Run Setup and Generate Sample Sheet Validate Single Input
3. NovaSeqDx Xp (NovaSeqDx v1.0)	1. Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.0)	Calculate Volumes Set Next Step Validate Inputs Flowcell Type and Single Pool Validate Unique Indexes (Not used. The core Clarity LIMS configuration for pooling handles this functionality.)
	2. Dilute, Denature, and ExAmp (NovaSeqDx v1.0)	Calculate Volumes Set Next Step Validate Inputs Flowcell Type
	3. Load to Flowcell (NovaSeqDx v1.0)	Validate Flowcell Barcode Validate Inputs and Selected Container Validate Run Setup and Generate Sample Sheet

Protocol	Step	Automation
4. AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.0)	1. AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.0)	Update Lane Number Set Next Steps (Not used. By default, the Sequencer API determines this functionality. If necessary, enable the automation and use it to override the next step behavior used by the Sequencer API.)

Terminology

As of Clarity LIMS v5, the term user-defined field (UDF) has been replaced with custom field in the user interface. However, the API resource is still called UDF.

There are two types of custom fields:

- Master step fields—Configured on master steps. Master step fields only apply to the following:
  - The master step on which the fields are configured.
  - The steps derived from those master steps.
- Global fields—Configured on entities (eg, submitted sample, derived sample, measurement, etc.). Global fields apply to the entire Clarity LIMS system.

Protocol 1: Run Format (NovaSeqDx v1.0)

This protocol sets the Loading Workflow Type and allows the choice of the appropriate Flowcell Type and Final Loading Concentration (pM). After the protocol, a routing script sends the normalized libraries to either the NovaSeqDx Standard (NovaSeqDx v1.0) or the NovaSeqDx Xp (NovaSeqDx v1.0) protocol.

This protocol contains one step: Define Run Format (NovaSeqDx v1.0).

Step 1. Define Run Format (NovaSeqDx v1.0)

Step input: Normalized Template Plate (NTP, normalized libraries)

Step output: None

Set Next Steps Automation

Automatically triggered on exit of the Record Details screen, this automation completes the following actions:

- Sets the next step for samples to REMOVE:

```
nextStep = ::REMOVE::
```


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- Checks NovaSeqDx Run Mode, Loading Workflow Type, and Flowcell Type. Only the NovaSeq Standard with the S2/S4 flow cell is supported for the NovaSeqDx Run Mode:

```
if (input.::NovaSeqDx Run Mode:: == ::DX::) {
    if (input.::Loading Workflow Type:: == ::NovaSeq Xp::) {
        fail(::Invalid option selected. NovaSeq Standard shall be selected for DX
        mode.::);
    };
    if (input.::Flowcell Type:: == ::SP:: || input.::Flowcell Type:: == ::S1::) {
        fail(::Invalid option selected. Only S2 or S4 flowcell shall be selected
        for DX mode.::);
    };
};
```

- Calculates the Minimum Molarity with the following formula:

```
input.::Minimum Molarity (nM):: = (5 * input.::Final Loading Concentration
(pM)::)/1000
```

- Checks Normalized Molarity value. For samples with no Normalized Molarity value (eg, empty value, not including 0), generates an error message informing that the field cannot be empty:

```
if (!input.hasValue(::Normalized Molarity (nM)::)) {
  fail(::The Normalized Molarity cannot be empty.::) ;
}
```

- Compares the Normalized Molarity value of each sample with the Minimum Molarity value. If the Normalized Molarity value is lower than the Minimum Molarity value, this sets the Loading Workflow Type of the sample to [Remove from workflow]. The automation also records a message in the Warning field for the sample:

```
else if (input.::Normalized Molarity (nM):: < input.::Minimum Molarity (nM)::) {
    input.::Warning:: = ::The Normalized Molarity is too low.::;
    input.::Loading Workflow Type:: = ::[Remove from workflow]::;
} else {
    input.::Warning:: = ::n/a::
}
```

At this point, the following options are available:

- Correct the NovaSeqDx Run Mode and Normalized Molarity value on the Record Details screen. Edit the Loading Workflow Type field and set it to NovaSeq Standard or NovaSeq Xp, as applicable.
- Complete the protocol without correcting the Normalized Molarity value. In this case, those samples are removed from the workflow.

Routing Script Automation

Automatically triggered on exit of the step, this automation invokes the changeWorkflow script, which routes step inputs appropriately.

- Samples with Loading Workflow Type field value = NovaSeq Standard are routed to the NovaSeqDx v1.0 workflow. Then, the samples are queued for the Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.0) step.
- Samples with Loading Workflow Type field value = NovaSeq Xp are routed to the NovaSeqDx v1.0 workflow. Then, the samples are queued for the Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.0) step.

Default automation command line is as follows.

```
bash -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar
-u {username} -p {password} -i {stepURI:v2} -l {compoundOutputFileLuid0}
script:changeWorkflow \
\
--FIELD_NAME 'Loading Workflow Type' \
--FIELD_VALUE 'NovaSeq Standard' \
--WORKFLOW 'NovaSeqDx v1.0' \
--STEP 'Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.0)' \
--INPUTS_OR_OUTPUTS 'INPUTS' \
\
--FIELD_NAME 'Loading Workflow Type' \
--FIELD_VALUE 'NovaSeq Xp' \--WORKFLOW 'NovaSeqDx v1.0' \
--STEP 'Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.0)' \
--INPUTS_OR_OUTPUTS 'INPUTS'"
```

Master Step Fields

There are four fields defined on the Define Run Format (NovaSeqDx v1.0) master step.

The following table lists field configuration details.

Table 82 Define Run Format (NovaSeqDx v1.0) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Comment	Multiline Text	• Required: No • Read Only: No	None

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Field Name	Field Type	Options	Additional Options and Dropdown Items
Flowcell Type	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• SP • S1 • S2 • S4
Instruction	Text	• Required: No • Read Only: Yes	• Default: Add Flowcell Type, Loading Workflow Type, and NovaSeqDx Run Mode
Loading Workflow Type	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp

Global Fields

The following table lists the global custom fields that are configured to display on the Define Run Format (NovaSeqDx v1.0) step.

Table 83 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
Adjusted Per Sample Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Final Loading Concentration (pM)	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 225 • 400 • Decimal Places Displayed: 0
Flowcell Type	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• SP • S1 • S2 • S4

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Field Name	Field Type	Options	Additional Options and Dropdown Items
Loading Workflow Type	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
Minimum Molarity (nM)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2
Normalized Molarity (nM)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2
NovaSeqDx Run Mode	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• DX • RUO
Per Sample Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Warning	Text Dropdown	• Required: No • Read Only: Yes • Custom Entries: Yes	• The Normalized Molarity (nM) is too low. • Not applicable

User Interaction

Refer to Run Define Run Format (NovaSeqDx v1.0) step section in *NovaSeq 6000Dx Integration v1.0 Validation and Troubleshooting* on page 244.

Protocol 2: NovaSeqDx Standard (NovaSeqDx v1.0)

Samples are routed to this protocol when their Loading Workflow Type value is set to NovaSeq Standard. Samples are pooled and added to a library tube in preparation for the NovaSeqDx Run.

At the end of this protocol, a routing script sends the library tube to the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.0) protocol.

This protocol contains the following two steps:

- Step 1: Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.0)
- Step 2: Dilute and Denature (NovaSeqDx v1.0)

Step 1: Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.0)

In this step, libraries are placed manually into a single pool. Resuspension buffer and reagents are added.

Create only one pool per step.

Step input: NTP (normalized libraries)

Step output: Bulk pool

Validate Unique Indexes automation is not used. The core Clarity LIMS configuration for pooling handles this functionality.

Validate Single NovaSeqDx Run Mode

The Validate Single NovaSeqDx Run Mode automation is triggered on entry of the step. The following automation checks that all input samples have the same NovaSeqDx Run Mode assigned:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar \
script:validate_same_analyte_udf_value_for_all_inputs \
-i {stepURI:v2} \
-u {username} \
-p {password} \
-l {compoundOutputFileLuid1} \
-f 'NovaSeqDx Run Mode'"
```

Validate Inputs Flowcell Type and Single Pool Automation

Automatically triggered on exit of the Pooling screen, this automation completes the following actions:

- Checks that all samples in the pool have the same Flowcell Type assigned to them.
- Checks that only one pool has been created.
- Changes the value of error and logging messages to reference the type of pool passed as bulk pool.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar \
script:validate_flowcell_for_input_pools \
-i {stepURI:v2} \
-u {username} \
-p {password} \
-l {compoundOutputFileLuid1} \
-validateSingleOutput true \
-poolType bulk"
```

Calculate Volumes Automation

Automatically triggered when you select the Calculate Volumes button on the Record Details screen, this automation completes the following actions:

- Calculates the number of samples in the pool.

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: + 1
```

- Sets the value of the Bulk Pool Volume (µl), and PhiX Volume (µl) field based on the selected Flowcell Type.

```
step.::PhiX Volume (ul):: = !step.hasValue(::% PhiX (2.5nM) Spike-In::) ? 0 :
step.::% PhiX (2.5nM) Spike-In::;
if (input.::Flowcell Type:: == ::SP::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 100
+ 30;
step.::PhiX Volume (ul):: *= 0.8
};
if (input.::Flowcell Type:: == ::S1::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 100
+ 30;
step.::PhiX Volume (ul):: *= 0.8
};
if (input.::Flowcell Type:: == ::S2::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 150
+ 30;
step.::PhiX Volume (ul):: *= 1.1
};
if (input.::Flowcell Type:: == ::S4::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 310
+ 30;
step.::PhiX Volume (ul):: *= 2.1
};
if (step.::PhiX Volume (ul):: == 0) {
step.::PhiX Volume (ul):: = ::::
};
```

- Calculates the Per Sample Volume (µl) to be added to the pool.

```
input.::Per Sample Volume (ul):: = ( ( (input.::Final Loading Concentration
(pM):: * 5 / 1000) / input.::Normalized Molarity (nM):: ) * step.::Bulk Pool
Volume (ul):: ) / step.::Number of Samples in Pool::
```

> For accurate pipetting of each sample in a pool for sequencing, the Per Sample Volume (µl) value must be equal to or higher than the Minimum Per Sample Volume (µl). The default value (set at 5) can be edited.

Assuming the default Minimum Per Sample Volume (µl) value of 5, this automation completes the following steps for a given batch:

1. If the smallest Per Sample Volume (µl) value is less than 5, automatically assigns a value of 5 to the Adjusted Per Sample Volume (µl) field of the sample.

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2. Adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

- Calculates the Total Sample Volume (µl) field value.

```
step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: +
input.::Adjusted Per Sample Volume (ul)::
```

- If the Total Sample Volume is less than the Bulk Pool Volume, this automation calculates the RSB Volume (µl) field value.

```
if (step.::Total Sample Volume (ul):: >= step.::Bulk Pool Volume (ul)::) {
output.::RSB Volume (ul):: = 0
}
else {
output.::RSB Volume (ul):: = step.::Bulk Pool Volume (ul):: - step.::Total
Sample Volume (ul)::
} ;
```

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs.

```
output.::Flowcell Type:: = input.::Flowcell Type:: ;
output.::Loading Workflow Type:: = input.::Loading Workflow Type:: ;
```

- Sets the Volume of Pool to Denature (µl) value a calculates the NaOH Volume (µl) and Tris-HCl Volume (µl) values based on the Flowcell Type.

```
if ( input.::Flowcell Type:: == ::SP:: ) {
output.::Volume of Pool to Denature (ul):: = 100 ;
output.::NaOH Volume (ul):: = 25 ;
output.::Tris-HCl Volume (ul):: = 25
} ;
if ( input.::Flowcell Type:: == ::S1:: ) {
output.::Volume of Pool to Denature (ul):: = 100 ;
output.::NaOH Volume (ul):: = 25 ;
output.::Tris-HCl Volume (ul):: = 25
} ;
if ( input.::Flowcell Type:: == ::S2:: ) {
output.::Volume of Pool to Denature (ul):: = 150 ;
output.::NaOH Volume (ul):: = 37.50 ;
output.::Tris-HCl Volume (ul):: = 37.50
} ;
if ( input.::Flowcell Type:: == ::S4:: ) {
output.::Volume of Pool to Denature (ul):: = 310 ;
output.::NaOH Volume (ul):: = 77.50 ;
output.::Tris-HCl Volume (ul):: = 77.50
```


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```
}
```

- Uses the `NovaSeq_Standard_Bulk_Pool1.csv`, `NovaSeq_Standard_Bulk_Pool2.csv`, and `NovaSeq_Standard_Bulk_Pool3.csv` template files to generate a single CSV file containing information about the pool and the samples it contains. The generated file is available for download in the Files section of the Record Details milestone in the Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.0) step.

```
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} \
   script:driver_file_generator \
      -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Standard_
      Bulk_Pool1.csv \
      -o 1.csv \
   script:driver_file_generator \
      -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Standard_
      Bulk_Pool2.csv \
      -o 2.csv \
   script:driver_file_generator \
      -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Standard_
      Bulk_Pool3.csv \
      -o 3.csv \
&& cat 1.csv 2.csv 3.csv > {compoundOutputFileLuid0}.csv
```

- Resets the Total Sample Volume (µl) and Number of Samples in Pool field values so that the automation is idempotent.

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: - 1;
step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: -
input.::Adjusted Per Sample Volume (ul)::
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, the following automation sets the next step for samples to ADVANCE, advancing them to the Dilute and Denature (NovaSeqDx v1.0) step in the protocol:

```
nextStep = ::ADVANCE::
```

The following automation copies the NovaSeqDx Run Mode values from the step inputs to the step outputs:

```
output.::NovaSeqDx Run Mode:: = input.::NovaSeqDx Run Mode::
```

Master Step Fields

There are seven fields defined on the Make Bulk Pool for NovaSeq Standard (NovaSeqDx v1.0) step. The following table lists configuration details.

Table 84 Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.0) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
% PhiX (2.5 nM) Spike-In	Numeric	Required: No Read Only: No	Range = 0–100 Decimal Places Displayed: 0
Bulk Pool Volume (µl) (for calculation purposes, not displayed)	Numeric	Required: No Read Only: No	Decimal Places Displayed: 2
Number of Flowcells to Sequence	Numeric	Required: Yes Read Only: No	Range = 1–10 Decimal Places Displayed: 0
Minimum Per Sample Volume (µl)	Numeric	Required: Yes Read Only: No	Decimal Places Displayed: 2 Default • 5
Number of Samples in Pool (for calculation purposes, not displayed)	Numeric	Required: No Read Only: No	Decimal Places Displayed: 0 Default • 0
PhiX Volume (µl) (for calculation purposes, not displayed)	Numeric	Required: No Read Only: No	Decimal Places Displayed: 2
Total Sample Volume (µl) (for calculation purposes, not displayed)	Numeric	Required: No Read Only: No	Decimal Places Displayed: 2 Default • 0

Global Fields

The following table lists the global custom fields that are configured to display on the Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.0) step.

Table 85 Global Custom Fields Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
Flowcell Type	Text Dropdown	Required: Yes Read Only: No Custom Entries: No	Presets • SP • S1 • S2 • S4
Loading Workflow Type	Text Dropdown	Required: Yes Read Only: No Custom Entries: No	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
NaOH Volume (µl)	Numeric	Required: No Read Only: Yes	Decimal Places Displayed: 2
RSB Volume (µl)	Numeric	Required: No Read Only: Yes	Decimal Places Displayed: 2
Tris-HCl Volume (µl)	Numeric	Required: No Read Only: Yes	Decimal Places Displayed: 2
Volume of Pool to Denature (µl) (Used in Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.0) step only. Displays on Record Details screen and in the generated CSV file.)	Numeric	Required: No Read Only: Yes	Decimal Places Displayed: 0

User Interaction

Refer to Run Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.0) step section in *NovaSeq 6000Dx Integration v1.0 Validation and Troubleshooting* on page 244.

Step 2: Dilute and Denature (NovaSeqDx v1.0)

In this step, the addition of NaOH, Tris-HCl, and Resuspension Buffer (RSB) denatures and dilutes pooled samples. Manually place the pooled samples into the library tube for the NovaSeqDx Run.

In addition, this step validates the run setup information and generates the sample sheet file.

Step input: Bulk pool

Step output: Library tube

Validate Single Input Automation

Automatically triggered at the beginning of the step, this automation checks that there is only one container input to the step.

```
script:validateSampleCount -min 1 -max 1
```

Validate Library Tube Barcode Automation

Automatically triggered on exit of the Placement screen, this automation completes the following actions:

- Validates the library tube barcode to ensure it conforms to the following barcode masks:
  - DX mode: [A-Z]{2}[0-9]{7}-[A-Z]{5}
  - RUO mode: [A-Z]{2}[0-9]{7}-[A-Z]{3}

```
if ((input.::NovaSeqDx Run Mode:: == ::DX:: && !output.container.name.matches(::
[A-Z]{2}[0-9]{7}-[A-Z]{5}::)) || (input.::NovaSeqDx Run Mode:: == ::RUO:: &&
!output.container.name.matches(::[A-Z]{2}[0-9]{7}-[A-Z]{3}::))) {
fail(::Invalid Library Tube Barcode. Please verify and try again.::)
}
```

- Copies Flowcell Type and Loading Workflow Type field values from step inputs to outputs:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ;
output.::Loading Workflow Type:: = input.::Loading Workflow Type::
```

Validate Run Setup and Generate Sample Sheet Automation

Triggered by a button on the Record Details screen, this automation completes the following actions:

> The script definition portion of Validate Run Setup has been moved into the Validation Script custom field due to the 4000 character limit. If modification is required on Validate Run Setup script, perform the changes directly on the Validation Script custom field default value.

- Copies the Flowcell Type from the step input to the Run Mode field (hidden):

```
step.::Run Mode:: = input.::Flowcell Type::
```

- Validates the parameters entered on the Record Details screen, which is used to set up the run and generate the sample sheet file. The parameters are as follows. For more information, refer to the *Respond to Validation / Recipe Request Call from Instrument* section of *NovaSeq 6000Dx Run* on page 231.
  - Experiment Name only contains alphanumeric, dash, or underscore characters. Spaces are not permitted.

  - When Workflow Type = No Index, both Index Read 1 and Index Read 2 must be zero.
  - When Workflow Type = Single Index, Index Read 1 must be greater than zero, and Index Read 2 must be zero.
  - When Workflow Type = Dual Index, both Index Read 1 and Index Read 2 must be greater than zero.

```
if(!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) {
fail(::Experiment Name contains prohibited characters. Allowed characters are:
a-z, A-Z, 0-9, -, and _::)
};
if (step.::Workflow Type:: == ::No Index::) {
if (step.::Index Read 1:: != 0 || step.::Index Read 2:: != 0) {
fail(::Index Read 1 and Index Read 2 must be 0 if No Index is selected.::)
}
} else if (step.::Workflow Type:: == ::Single Index::) {
if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) {
fail(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if Single
Index is selected.::)
}
} else {
if (step.::Index Read 1:: == 0 || step.::Index Read 2:: == 0) {
fail(::Index Read 1 and Index Read 2 must be greater than 0 if Dual Index is
selected.::)
}
};
```

- Checks the Paired End and Read 2 Cycles field values as follows.
  - If Paired End = True, both Read 1 Cycles and Read 2 Cycles must be greater than 0.
  - If Paired End = False, Read 2 Cycles must be zero.

```
if (step.::Paired End::.toBoolean()) {
    if (step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) {
        fail(::Read 1 Cycles and Read 2 Cycles must be greater than 0 if Paired
        End is True.::)
    }
} else {
    if (step.::Read 2 Cycles:: != 0) {
        fail(::Read 2 Cycles must be 0 if Paired End is False.::)
    }
};
```

- Checks the Flowcell field value as follows.

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  - If Flowcell Type value is not SP, checks that the values of Read 1 Cycles and Read 2 Cycles are each 151 or less. If the value is greater than 151, an error message is generated.

```
if (input.::Flowc ell Type::!=::SP:: && step.::Read 1 Cycles:: > 151) {
fail(::Read 1 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
};
if (input.::Flowcell Type::!=::SP:: && step.::Read 2 Cycles:: > 151) {
fail(::Read 2 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
};
```

- Validate Analysis Software Version to ensure it is a valid version string.

```
if (step.hasValue(::Analysis Software Version::) && !step.::Analysis Software
Version::.matches(::^(\\d+)(\\.\\d+)*$::)) {
fail(::Analysis Software Version contains prohibited characters. Allowed
characters are 0-9 and period. It shall start and end with numbers and separated
by single period e.g. 3.8.4::)
};
```

- Validate Override Cycles value to contain only Y, N, I, U, 0-9, and semicolon characters.

```
if (step.hasValue(::Override Cycles::) && !step.::Override Cycles::.matches(::
[YNIU0-9;]+::)) {
fail(::Override Cycles contains prohibited characters. Allowed characters are:
Y, N, I, U, 0-9 and ;. Example: N1Y150;I8;I7N1;Y141U10.::)
};
```

- Sets the next step for samples to REMOVE:

```
nextStep = ::REMOVE::
```

- Generates the V2 sample sheet and attaches it to the step. For more information, refer to *Sample Sheet Generation* on page 230.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeqDx_BCL2FASTQ_
Samplesheet_v2.csv \
-o {compoundOutputFileLuid0}.csv \
-q true \
-destLIMSID {compoundOutputFileLuid0} \
-l {compoundOutputFileLuid1}"
```

Routing Script Automation

Automatically triggered on exit of the step, this automation invokes the changeWorkflow script, which routes step outputs to the NovaSeqDx v1.0 workflow, and queues them for the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.0) step.

The default automation command line is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar
-u {username} -p {password} -i {stepURI:v2} -l {compoundOutputFileLuid1} script:changeWorkflow \
--FIELD_NAME 'N/A' \
--FIELD_VALUE 'N/A' \
--WORKFLOW 'NovaSeqDx v1.0' \
--STEP 'AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.0)' \
--INPUTS_OR_OUTPUTS 'OUTPUTS'"
```

Master Step Fields

There are 19 fields defined on the Dilute and Denature (NovaSeqDx v1.0) step. These fields are required for sample sheet and JSON file generation.

The following table lists field configuration details.

Table 86 Dilute and Denature (NovaSeqDx v1.0) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Analysis Software Version	Text	Required: No Read Only: No	
BaseSpace Sequence Hub Configuration	Text Dropdown	Required: Yes Read Only: No Custom Entries: No	Not Used Run Monitoring Only Run Monitoring and Storage
Custom Recipe Path	Text	Required: No Read Only: No	
Experiment Name	Text	Required: Yes Read Only: No	

Field Name	Field Type	Options	Additional Options and Dropdown Items
Index Read 1	Numeric Dropdown	Required: Yes Read Only: No Custom Entries: Yes	Range = 0–20 Decimal Places Displayed: 0 Presets • 0 • 6 • 8
Index Read 2	Numeric Dropdown	Required: Yes Read Only: No Custom Entries: Yes	Range = 0–20 Decimal Places Displayed: 0 Presets • 0 • 6 • 8
Output Folder	Text	Required: Yes Read Only: No	
Override Cycles	Text	Required: No Read Only: No	
Paired End	Text Dropdown	Required: Yes Read Only: No Custom Entries: No	Presets • True • False
Read 1 Cycle	Numeric Dropdown	Required: Yes Read Only: No Custom Entries: Yes	Range = 1–251 Decimal Places Displayed: 0 Presets • 251* • 151 • 101 • 51 *Value of 251 is only supported for SP flow cell type. For all other cell types, maximum value is 151.

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Field Name	Field Type	Options	Additional Options and Dropdown Items
Read 2 Cycle	Numeric Dropdown	Required: Yes Read Only: No Custom Entries: Yes	Range = 0–251 Decimal Places Displayed: 0 Presets • 251* • 151 • 101 • 51 *Value of 251 is only supported for SP flow cell type. For all other cell types, maximum value is 151.
Run Mode (Not displayed in user interface)	Text Dropdown	Required: No Read Only: Yes Custom Entries: No	Presets • SP • S1 • S2 • S4
Use Custom Index Read 1 Primer	Toggle Switch	Required: No Read Only: No	Default • None Set
Use Custom Read 1 Primer	Toggle Switch	Required: No Read Only: No	Default • None Set
Use Custom Read 2 Primer	Toggle Switch	Required: No Read Only: No	Default • None Set
Use Custom Recipe	Toggle Switch	Required: Yes Read Only: No	Default • No

Field Name	Field Type	Options	Additional Options and Dropdown Items
Validation Script	Multiline Text	Required: Yes Read Only: Yes	Default value is provided in the drop-down section that follows the table. Do not remove this field as it is used by Validate Run Setup and Generate Sample Sheet automation script.
Workflow	Text	Required: No Read Only: Yes	Default • GenerateFASTQ
Workflow Type	Text Dropdown	Required: Yes Read Only: No Custom Entries: No	Presets • No Index • Single Index • Dual Index • Custom

Validation Script

```
step.::Run Mode:: = input.::Flowcell Type::; if (!step.::Experiment
Name::.matches(::[a-zA-Z0-9-_]+::)) { fail(::Experiment Name contains prohibited
characters. Allowed characters are: a-z, A-Z, 0-9, -, and _::) }; if
(step.::Workflow Type:: == ::No Index::) { if (step.::Index Read 1:: != 0 ||
step.::Index Read 2:: != 0) { fail(::Index Read 1 and Index Read 2 must be 0 if
No Index is selected.::) } } else if (step.::Workflow Type:: == ::Single
Index::) { if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) { fail
(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if Single
Index is selected.::) } } else { if (step.::Index Read 1:: == 0 || step.::Index
Read 2:: == 0) { fail(::Index Read 1 and Index Read 2 must be greater than 0 if
Dual Index is selected.::) } }; if (step.::Paired End::.toBoolean()) { if
(step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) { fail(::Read 1
Cycles and Read 2 Cycles must be greater than 0 if Paired End is True.::) } }
else { if (step.::Read 2 Cycles:: != 0) { fail(::Read 2 Cycles must be 0 if
Paired End is False.::) } }; if (input.::Flowcell Type:: != ::SP:: &&
```

```
step.::Read 1 Cycles:: > 151) { fail(::Read 1 Cycles must not be larger than 151
if it is not SPrime Flowcell::) }; if (input.::Flowcell Type:: != ::SP:: &&
step.::Read 2 Cycles:: > 151) { fail(::Read 2 Cycles must not be larger than 151
if it is not SPrime Flowcell::) }; if (step.hasValue(::Analysis Software
Version::) && !step.::Analysis Software Version::.matches(::^(\\d+)
(\\.\\d+)*$::)) { fail(::Analysis Software Version contains prohibited
characters. Allowed characters are 0-9 and period. It shall start and end with
numbers and separated by single period e.g. 3.8.4::) }; if (step.hasValue
(::Override Cycles::) && !step.::Override Cycles::.matches(::[YNIU0-9;]+::)) {
fail(::Override Cycles contains prohibited characters. Allowed characters are:
Y, N, I, U, 0-9 and ;. Example: N1Y150;I8;I7N1;Y141U10.::) }; nextStep =
::REMOVE::
```

Global Fields

The following table lists the global custom fields that are configured to display on the Dilute and Denature (NovaSeqDx v1.0) step.

Table 87 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
Flowcell Type	Text Dropdown	Required: Yes Read Only: No Custom Entries: No	Presets • SP • S1 • S2 • S4
Loading Workflow Type	Text Dropdown	Required: Yes Read Only: No Custom Entries: No	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User Interaction

Refer to Run Dilute and Denature (NovaSeqDx v1.0) step section in *NovaSeq 6000Dx Integration v1.0 Validation and Troubleshooting* on page 244.

Protocol 3: NovaSeqDx Xp (NovaSeqDx v1.0)

Samples are routed to this protocol when their Loading Workflow Type value is set to NovaSeq Xp.

Samples are pooled and added to lanes on the NovaSeq flow cell type selected in the Define Run Format (NovaSeqDx v1.0) step. At the end of this protocol, the flow cell is sent to the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.0) protocol.

This protocol contains the following three steps:

- Step 1: Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.0)
- Step 2: Dilute, Denature, and ExAmp (NovaSeqDx v1.0)
- Step 3: Load to Flowcell (NovaSeqDx v1.0)

Step 1: Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.0)

Manually place libraries into a pool.

Create only one pool per step.

Step input: NTP (normalized libraries)

Step output: Bulk pool

Validate Unique Indexes automation is not used. The core Clarity LIMS configuration for pooling handles this functionality.

Validate Inputs Flowcell Type and Single Pool Automation

Automatically triggered on exit of the Pooling screen, this automation completes the following actions:

- Checks that all samples in the pool have the same Flowcell Type assigned to them.
- Checks that only one pool has been created.
- Changes the value of error and logging messages to reference the type of pool passed as 'bulk' pool.

```
/opt/gls/clarity/bin/java \
-jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar \
script:validate_flowcell_for_input_pools \
-i {stepURI:v2} \
-u {username} \
-p {password} \
-l {compoundOutputFileLuid1} \
-validateSingleOutput true \
-poolType bulk"
```

Calculate Volumes Automation

Triggered when the Calculate Volumes button is selected on the Record Details screen, this automation completes the following actions:

- Calculates the number of samples in the pool:

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: + 1
```

- Sets the value of the Bulk Pool Volume (µl) and PhiX Volume (µl) field, based on the selected Flowcell Type:

```
step.::PhiX Volume (ul):: = !step.hasValue(::% PhiX (0.25nM) Spike-In::) ? 0 :
step.::% PhiX (0.25nM) Spike-In::;
if (input.::Flowcell Type:: == ::SP::) {
step.::Bulk Pool Volume (ul):: = step.'Number of Lanes to Sequence' * 18 + 30;
step.::PhiX Volume (ul):: *= 1.9;
};
if (input.::Flowcell Type:: == ::S1::) {
step.::Bulk Pool Volume (ul):: = step.'Number of Lanes to Sequence' * 18 + 30;
step.::PhiX Volume (ul):: *= 1.9;
};
if (input.::Flowcell Type:: == ::S2::) {
step.::Bulk Pool Volume (ul):: = step.'Number of Lanes to Sequence' * 22 + 30;
step.::PhiX Volume (ul):: *= 1.9;
};
if (input.::Flowcell Type:: == ::S4::) {
step.::Bulk Pool Volume (ul):: = step.'Number of Lanes to Sequence' * 30 + 30;
step.::PhiX Volume (ul):: *= 2.2;
};
if (step.::PhiX Volume (ul):: == 0) {
step.::PhiX Volume (ul):: = ::::;
};
```

- Calculates the Per Sample Volume (µl) to be added to the pool:

```
input.::Per Sample Volume (µl)::= (((input.::Final Loading Concentration (pM)::*
5/ 1000) / input.::Normalized Molarity (nM)::) * step.::Bulk Pool Volume (µl)::)
/ step.::Number of Samples in Pool::
```



To ensure accurate pipetting of each sample in a pool for sequencing, the Per Sample Volume (µl) value must be equal to or higher than the Minimum Per Sample Volume (µl). The default value (set at 5) can be edited.

Assuming the default Minimum Per Sample Volume (µl) value of 5, completes the following steps for a given batch:

1. If the smallest Per Sample Volume (µl) value is less than 5, automatically assigns a value of 5 to the sample Adjusted Per Sample Volume (µl) field.
2. Adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

- Calculates the Total Sample Volume (µl) field value:

```
step.::Total Sample Volume (µl):: = step.::Total Sample Volume (µl):: +
input.::Adjusted Per Sample Volume (µl)::
```

- If the Total Sample Volume is less than the Bulk Pool Volume, this automation calculates the RSB Volume (µl) field value:

```
if (step.::Total Sample Volume (ul):: >= step.::Bulk Pool Volume (ul)::) {
output.::RSB Volume (ul):: = 0
}
else {
    output.::RSB Volume (ul):: = step.::Bulk Pool Volume (ul):: - step.::Total
    Sample Volume (ul)::
} ;
```

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs:

```
output.::Flowcell Type::= input.::Flowcell Type::;output.::Loading Workflow
Type::= input.::Loading Workflow Type::;
```

- Uses the `NovaSeq_Xp_Bulk_Pool.csv` and `NovaSeq_Xp_Bulk_Pool2.csv` template files to generate a single CSV file containing information about the bulk pool and the samples it contains. The generated file is available for download on the Step Setup screen of the Dilute, Denature, and ExAmp (NovaSeqDx v1.0) step.

```
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} \
    script:driver_file_generator \
       -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_Bulk_
       Pool.csv \
       -o {compoundOutputFileLuid0}.csv \
    script:driver_file_generator \
       -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_Bulk_
       Pool2.csv \
       -o append.csv \
&& cat append.csv >> {compoundOutputFileLuid0}.csv"
```

- Resets the Total Sample Volume (µl) and Number of Samples in Pool field values so that the automation is idempotent.

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: - 1;
step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: -
input.::Adjusted Per Sample Volume (ul)::
```


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Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation completes the following actions:

- Copies the Flowcell Type values from the step inputs to the step outputs.
- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ; nextStep = ::ADVANCE::
```

Master Step Fields

There are seven fields defined on the Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.0) step.

Table 88 Make Bulk Pool for NovaSeq Xp (NovaSeqDx v1.0) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
% PhiX (0.25nM) Spike-in	Numeric	• Required: No • Read Only: No	• Range From: 0 to 100
Bulk Pool Volume (µl) (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2
Minimum Per Sample Volume (µl)	Numeric	• Required: Yes • Read Only: No	• Default: 5 • Decimal Places Displayed: 2
Number of Lanes to Sequence	Numeric	• Required: Yes • Read Only: No	• Decimal Places Displayed: 0
Number of Samples in Pool (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Default: 0 • Decimal Places Displayed: 0
PhiX Volume (µl) (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2
Total Sample Volume (µl) (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Default: 0 • Decimal Places Displayed: 0

Global Fields

The following table lists the global custom fields that are configured to display on the Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.0) step.

Table 89 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
RSB Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Flowcell Type	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• SP • S1 • S2 • S4
Loading Workflow Type	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User Interaction

Refer to Run Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.0) step section in *NovaSeq 6000Dx Integration v1.0 Validation and Troubleshooting* on page 244.

Step 2: Dilute, Denature and ExAmp (NovaSeqDx v1.0)

In this step, the addition of DPX, NaOH, Tris-HCl, and RSB denatures and dilutes pooled samples. Manually create working pools based on the number of lanes that you want to sequence.

Step input: Bulk pool

Step output: Working pool - variable number (choose how many working pools to create per bulk pool)

Validate Inputs Flowcell Type Automation

Automatically triggered at the beginning of the step, this automation invokes the `validate_flowcell_for_input_pools` script. This script completes the following actions:

- Checks the inputs to the step and validates that the Flowcell Type field is set to a valid value (SP, S1, S2, or S4). This automation also checks that all inputs have the same value for this field.
- The container type selected matches the value in the Flowcell Type field.

```
bash -l -c "/opt/gls/clarity/bin/java \
```

```
-jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar \
script:validate_flowcell_for_input_pools \
-i {stepURI:v2} \
-u {username} \
-p {password} \
-l {compoundOutputFileLuid1}"
```

Calculate Volumes Automation

Automatically triggered on entry to of the Record Details screen, this automation sets the value of the following fields based on the Flowcell Type:

- BP Aliquot Volume (µl)
- NaOH Volume (µl)
- Tris-HCl Volume (µl)
- DPX1 Volume (µl)
- DPX2 Volume (µl)
- DPX3 Volume (µl)
- Mastermix per Lane (µl)

```
if ( input.::Flowcell Type:: == ::SP:: ) {
output.::BP Aliquot Volume (µl):: = 18 ;
output.::NaOH Volume (µl):: = 4.50 ;
output.::Tris-HCl Volume (µl):: = 4.50 ;
step.::DPX1 Volume (µl):: = 126 ;
step.::DPX2 Volume (µl):: = 18 ;
step.::DPX3 Volume (µl):: = 66 ;
output.::Mastermix per Lane (µl):: = 63
};

if (input.::Flowcell Type:: == ::S1::) {
output.::BP Aliquot Volume (µl):: = 18;
output.::NaOH Volume (µl):: = 4.50;
output.::Tris-HCl Volume (µl):: = 4.50;
step.::DPX1 Volume (µl):: = 126;
step.::DPX2 Volume (µl):: = 18;
step.::DPX3 Volume (µl):: = 66;
output.::Mastermix per Lane (µl):: = 63
};
if (input.::Flowcell Type:: == ::S2::) {
```

```
output.::BP Aliquot Volume (µl):: = 22;
output.::NaOH Volume (µl):: = 5.50;
output.::Tris-HCl Volume (µl):: = 5.50;
step.::DPX1 Volume (µl):: = 126;
step.::DPX2 Volume (µl):: = 18;
step.::DPX3 Volume (µl):: = 66;
output.::Mastermix per Lane (µl):: = 77
};
if (input.::Flowcell Type:: == ::S4::) {
output.::BP Aliquot Volume (µl):: = 30;
output.::NaOH Volume (µl):: = 7.50;
output.::Tris-HCl Volume (µl):: = 7.50;
step.::DPX1 Volume (µl):: = 315;
step.::DPX2 Volume (µl):: = 45;
step.::DPX3 Volume (µl):: = 165;
output.::Mastermix per Lane (µl):: = 105
};
```

The automation also completes the following actions:

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs:

```
output.::Flowcell Type::= input.::Flowcell Type::;
output.::Loading Workflow Type::= input.::Loading Workflow Type::;
```

- Uses the `NovaSeq_Xp_Working_Pool.csv` and `NovaSeq_Xp_Working_Pool2.csv` template files to generate a single CSV file. This file contains information about the DPX volume and the volume of BP Aliquot, Mastermix, NaOH, and Tris-HCl to add per working pool. The generated file is available for download on the Step Setup screen of the Dilute, Denature & ExAmp (NovaSeqDx v1.0) step.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} \
   script:driver_file_generator \
      -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_
      Working_Pool.csv \
      -o {compoundOutputFileLuid0}.csv \
   script:driver_file_generator \
      -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_
      Working_Pool2.csv \
      -o append.csv \
&& cat append.csv >> {compoundOutputFileLuid0}.csv"
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation:

- Copies the Flowcell Type values from the step inputs to the step outputs.
- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ; nextStep = ::ADVANCE::
```

Master Step Fields

There are three fields defined on the Dilute, Denature & ExAmp (NovaSeqDx v1.0) step. A script sets these field values. They are not editable while running the step.

The following table lists field configuration details.

Table 90 Dilute, Denature & ExAmp (NovaSeq v1.0) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
DPX1 Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0
DPX2 Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0
DPX3 Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0

Global Fields

The following table lists the global custom fields that are configured to display on the Dilute, Denature & ExAmp (NovaSeqDx v1.0) step.

Table 91 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
BP Aliquot Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0

For Research Use Only. Not for use in diagnostic procedures.

Field Name	Field Type	Options	Additional Options and Dropdown Items
Flowcell Type	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• SP • S1 • S2 • S4
Loading Workflow Type	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
Mastermix per Lane (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0
NaOH Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Tris-HCl Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2

User Interaction

Refer to Run Dilute, Denature & ExAmp (NovaSeqDx v1.0) step section in *NovaSeq 6000Dx Integration v1.0 Validation and Troubleshooting* on page 244.

Step 3: Load to Flowcell (NovaSeqDx v1.0)

In this step, scan the flow cell barcode into the Clarity LIMS and manually place the working pools into the lanes of the flow cell for the NovaSeqDx run. This step validates the run setup information and generates the sample sheet file.

Step input: Working pool

Step output: Flow cell (output containers: SP, S1, and S2 with 2 lanes, and S4 with 4 lanes)

Validate Inputs and Selected Container Automation

Automatically triggered at the beginning of the step, this automation invokes the validate_flowcell_for_input_pools and validateSelectedContainer scripts. These scripts validate the step inputs and the container selected by the user, as follows.

- Checks that the Flowcell Type field has been set to a valid value (SP, S1, S2, or S4) and that all inputs have the same value.

- Checks that the number of outputs matches the number of lanes on the selected flow cell type. If validation fails, an error message informs that the number of working pools does not match the number of lanes available on the flow cell.

The following table defines the number of samples allowed:

Flow Cell Type	Number of Working Pools
SP	2
S1	2
S2	2
S4	4

- Checks that the container type selected on entry to the Placement screen matches the value in the Flowcell Type field. If validation fails, an error message displays.

```
bash -l -c "/opt/gls/clarity/bin/java \
-jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar
\script:validate_flowcell_for_input_pools \
  -i {stepURI:v2} \
  -u {username} \
  -p {password} \
  -l {compoundOutputFileLuid1} \
  -validateSelectedContainer true"
```

Validate Flowcell Barcode Automation

Automatically triggered on exit of the Placement screen, this automation validates the flow cell barcode scanned into the Clarity LIMS by the user using the following logic:

```
if (input.::Flowcell Type:: == ::SP:: && !output.container.name.matches( ::[A-
Z0-9]{5}DRX[XY2357]:: ) ) {
fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: )
};
if (input.::Flowcell Type:: == ::S1:: && !output.container.name.matches( ::[A-
Z0-9]{5}DRX[XY2357]:: ) ) {
fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: )
};
if (input.::Flowcell Type:: == ::S2:: && !output.container.name.matches( ::[A-
Z0-9]{5}DMX[XY2357]:: ) ) {
fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: )
};
```

```
if (input.::Flowcell Type:: == ::S4:: && !output.container.name.matches( ::[A-Z0-9]{5}DSX[XY2357]:: ) ) {
fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: )
};
```

- Copies the Flowcell Type and Loading Workflow Type field values from step inputs to outputs:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ;
output.::Loading Workflow Type:: = input.::Loading Workflow Type::
```

Validate Run Setup and Generate Sample Sheet Automation

The script definition portion of Validate Run Setup has been moved into the Validation Script custom field due to the 4000 character limitation.

- If modification is required to the Validate Run Setup script, perform the changes directly on Validation Script custom field default value.
- The script definition portion of Generate Sample Sheet is unchanged.

Automatically triggered by selecting a button on the Record Details screen, this automation:

- Copies the Flowcell Type from the step input to the Run Mode field (hidden):

```
step.::Run Mode:: = input.::Flowcell Type::
```

- Validates the parameters entered on the Record Details screen, which is used to set up the run and generate the sample sheet file.
  - When Workflow Type = No Index, both Index Read 1 and Index Read 2 must be 0.
  - When Workflow Type = Single Index, Index Read 1 must be greater than 0 and Index Read 2 must be 0.
  - When Workflow Type = Dual Index, both Index Read 1 and Index Read 2 must be greater than 0.

```
if(!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) {
fail(::Experiment Name contains prohibited characters. Allowed characters are: a-z, A-Z, 0-9, -, and _::)
};
if (step.::Workflow Type:: == ::No Index::) {
if (step.::Index Read 1:: != 0 || step.::Index Read 2:: != 0) {
fail(::Index Read 1 and Index Read 2 must be 0 if No Index is selected.::)
}
} else if (step.::Workflow Type:: == ::Single Index::) {
if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) {
fail(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if Single Index is selected.::)
}
```


For Research Use Only. Not for use in diagnostic procedures.

```
} else {
if (step.::Index Read 1:: == 0 || step.::Index Read 2:: == 0) {
fail(::Index Read 1 and Index Read 2 must be greater than 0 if Dual Index is
selected.::)
}
};
```

- Checks the Paired End and Read 2 Cycles field values.
  - When Paired End = True, both Read 1 Cycles and Read 2 Cycles must be greater than 0.
  - When Paired End = False, Read 2 Cycles must be 0.

```
if (step.::Paired End:.toBoolean()) {
   if (step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) {
      fail(::Read 1 Cycles and Read 2 Cycles must be greater than 0 if Paired
      End is True.::)
   }
} else {
   if (step.::Read 2 Cycles:: != 0) {
      fail(::Read 2 Cycles must be 0 if Paired End is False.::)
   }
};
```

- Checks the Flowcell field value.
  - If Flowcell Type value is not SP, checks that the value of both Read 1 Cycles and Read 2 Cycles is 151 or less. If the value is greater than 151, an error message displays.

```
if (input.::Flowcell Type::!=::SP:: && step.::Read 1 Cycles:: > 151) {
fail(::Read 1 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
}
if (input.::Flowcell Type::!=::SP:: && step.::Read 2 Cycles:: > 151) {
fail(::Read 2 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
}
```

- Validate Analysis Software Version

```
if (step.hasValue(::Analysis Software Version::) && !step.::Analysis Software
Version::.matches(::^(\\d+)(\\.\\d+)*$::)) {
fail(::Analysis Software Version contains prohibited characters. Allowed
characters are 0-9 and period. It shall start and end with numbers and separated
by single period e.g. 3.8.4::)
};
```

- Checks Override Cycles field value. This field contains only Y, N, I, U, 0–9, and semicolon characters.

For Research Use Only. Not for use in diagnostic procedures.

```
if (step.hasValue(::Override Cycles::) && !step.::Override Cycles::.matches(::
[YNIU0-9;]+::)) {
fail(::Override Cycles contains prohibited characters. Allowed characters are:
Y, N, I, U, 0-9 and ;. Example: N1Y150;I8;I7N1;Y141U10.::)
};
```

- Sets the next step for samples to ADVANCE, advancing them to the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.0) step.

```
nextStep = ::ADVANCE::
```

- Generates the sample sheet and attaches it to the step. For more information, refer to *Sample Sheet Generation* on page 230

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeqDx_BCL2FASTQ_
Samplesheet_v2.csv \
-o {compoundOutputFileLuid0}.csv \
-q true \
-destLIMSID {compoundOutputFileLuid0} \
-l {compoundOutputFileLuid1}
```

Master Step Fields

The following table lists the field configuration details for the fields defined on the Load to Flowcell (NovaSeqDx v1.0) step.

Table 92 Load to Flowcell (NovaSeqDx v1.0) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Analysis Software Version	Text	• Required: No • Read Only: No	
BaseSpace Sequence Hub Configuration	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• Not Used • Run Monitoring Only • Run Monitoring and Storage

Field Name	Field Type	Options	Additional Options and Dropdown Items
Custom Recipe Path	Text	• Required: No • Read Only: No	
Experiment Name	Text	• Required: Yes • Read Only: No	
Index Read 1	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 0 • 6 • 8 • Range From: 0 to 20 • Decimal Places Displayed: 0
Index Read 2	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 0 • 6 • 8 • Range From: 0 to 20 • Decimal Places Displayed: 0
Library Tube Barcode	Text	• Required: Yes • Read Only: No	
Output Folder	Text	• Required: Yes • Read Only: No	
Override Cycles	Text	• Required: No • Read Only: No	
Paired End	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• True • False

Field Name	Field Type	Options	Additional Options and Dropdown Items
Read 1 Cycles	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 251* • 151 • 101 • 51 • Range From: 1 to 251 • Decimal Places Displayed: 0 *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Read 2 Cycles	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 251* • 151 • 101 • 51 • Range From: 0 to 251 • Decimal Places Displayed: 0 *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Run Mode (not displayed in user interface)	Text Dropdown	• Required: No • Read Only: Yes • Custom Entries: No	• SP • S1 • S2 • S4
Use Custom Index Read 1 Primer	Toggle Switch	• Required: No • Read Only: No	• Default: None Set
Use Custom Read 1 Primer	Toggle Switch	• Required: No • Read Only: No	• Default: None Set

Field Name	Field Type	Options	Additional Options and Dropdown Items
Use Custom Read 2 Primer	Toggle Switch	• Required: No • Read Only: No	• Default: None Set
Use Custom Recipe	Toggle Switch	• Required: Yes • Read Only: No	• Default: No
Validation Script	Multiline Text	• Required: Yes • Read Only: Yes	• Default value provided in the drop-down section following the table. Do not remove this field as it is used by Validate Run Setup and Generate Sample Sheet automation script.
Workflow	Text	• Required: No • Read Only: Yes	• Default: GenerateFASTQ
Workflow Type	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• No Index • Single Index • Dual Index • Custom

Validation Script

```
step.::Run Mode:: = input.::Flowcell Type::; if (!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) { fail(::Experiment Name contains prohibited characters. Allowed characters are: a-z, A-Z, 0-9, -, and _::) }; if (step.::Workflow Type:: == ::No Index::) { if (step.::Index Read 1:: != 0 || step.::Index Read 2:: != 0) { fail(::Index Read 1 and Index Read 2 must be 0 if No Index is selected.::) } } else if (step.::Workflow Type:: == ::Single Index::) { if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) { fail(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if Single Index is selected.::) } } else { if (step.::Index Read 1:: == 0 || step.::Index Read 2:: == 0) { fail(::Index Read 1 and Index Read 2 must be greater than 0 if Dual Index is selected.::) } }; if (step.::Paired End::.toBoolean()) { if
```

```
(step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) { fail(::Read 1
Cycles and Read 2 Cycles must be greater than 0 if Paired End is True.::) } }
else { if (step.::Read 2 Cycles:: != 0) { fail(::Read 2 Cycles must be 0 if
Paired End is False.::) }}; if (input.::Flowcell Type:: != ::SP:: && step.::Read
1 Cycles:: > 151) { fail(::Read 1 Cycles must not be larger than 151 if it is
not SPrime Flowcell::) }; if (input.::Flowcell Type:: != ::SP:: && step.::Read 2
Cycles:: > 151) { fail(::Read 2 Cycles must not be larger than 151 if it is not
SPrime Flowcell::) }; if (step.hasValue(::Analysis Software Version::) &&
!step.::Analysis Software Version::.matches(::^(\\d+)(\\.\\d+)*$::)) { fail
(::Analysis Software Version contains prohibited characters. Allowed characters
are 0-9 and period. It shall start and end with numbers and separated by single
period e.g. 3.8.4::) }; if (step.hasValue(::Override Cycles::) &&
!step.::Override Cycles::.matches(::[YNIU0-9;]+::)) { fail(::Override Cycles
contains prohibited characters. Allowed characters are: Y, N, I, U, 0-9 and ;.
Example: N1Y150;I8;I7N1;Y141U10.::) }; step.::Settings Header:: = step.hasValue
(::UMI - Read 1 Length::) || step.hasValue(::UMI - Read 2 Length::) ? ::
[Settings]:: : ::::; nextStep = ::REMOVE::
```

Global Fields

The following table lists the global custom fields that are configured to display on the Load to Flowcell (NovaSeqDx v1.0) step.

Table 93 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured in Analyte on Derived Sample			
Flowcell Type	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• SP • S1 • S2 • S4
Loading Workflow Type	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User Interaction

For more information, refer to the Run Load to Flowcell (NovaSeqDx v1.0) step section in *NovaSeq 6000Dx Integration v1.0 Validation and Troubleshooting* on page 244.

Protocol 4: AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.0)

This final protocol contains one fully automated step, AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.0).

This step is fully automated. Do not add samples to the Ice Bucket or start the step manually. If steps are manually started, the sequencing service does not update the samples correctly.

Step 1: AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.0)

Step input: Library tube from NovaSeqDx Standard or flow cell from NovaSeqDx Xp protocol

Step output: Result file/measurement

In this step, pooled samples are sequenced on the NovaSeq 6000Dx instrument and the run metrics are recorded in Clarity LIMS.

Set Next Steps Automation

If necessary, override the default next step behavior used by the Sequencer API by performing the following steps:

1. Enable the automation and use it to set custom next step behavior and generate a log file. By default, the automation sets the next step to ADVANCE and the samples complete the protocol.
2. Configure the automation to trigger automatically on exit of the Record Details screen.
3. Disable or configure auto-complete behavior by setting the values of the run.autoComplete and autoCompleteOnlyAtSuccess properties. For details, refer to *Configure Autocomplete Properties* on page 242.

Update Lane Number Automation

This automation triggers automatically upon entry to the Record Details screen.

Do not disable or modify this automation to ensure the Lane Number displays properly.

Master Step Fields

The following table shows the master step fields that are configured on the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.0) step.

Table 94 Clarity LIMS Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Current Cycle	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0
Current Read	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0
Firmware Version	Text	• Required: No • Read Only: Yes	
Flow Cell Expiration Date	Date	• Required: No • Read Only: Yes	
Flow Cell ID	Text	• Required: No • Read Only: Yes	
Flow Cell Lot Number	Text	• Required: No • Read Only: Yes	
Flow Cell Mode	Text	• Required: No • Read Only: Yes	
Flow Cell Part Number	Text	• Required: No • Read Only: Yes	
Flow Cell Side	Text	• Required: No • Read Only: Yes	
Instrument Control Software Version	Text	• Required: No • Read Only: Yes	
Instrument ID	Text	• Required: No • Read Only: Yes	
Instrument Type	Text	• Required: No • Read Only: Yes	
Lane Counter	Numeric	• Required: No • Read Only: Yes	• Default: 1 • Hidden
Loading Workflow Type	Text	• Required: No • Read Only: Yes	
Output Folder	Text	• Required: No • Read Only: Yes	

For Research Use Only. Not for use in diagnostic procedures.

Field Name	Field Type	Options	Additional Options and Dropdown Items
RTA Version	Text	• Required: No • Read Only: Yes	
Run Completion Date	Text	• Required: No • Read Only: Yes	• Hidden
Run ID	Text	• Required: Yes • Read Only: Yes	
Run Status	Text	• Required: No • Read Only: Yes	
Sequencing Log	Multiline Text	• Required: No • Read Only: Yes	

Global Fields

The following table shows the global custom fields that capture the run metrics in Clarity LIMS.

Table 95 Custom Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
% Aligned R1	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
% Aligned R2	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
% Bases >=Q30 R1	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
% Bases >=Q30 R2	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
% Error Rate R1	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
% Error Rate R2	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
% Phasing R1	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 3

Field Name	Field Type	Options	Additional Options and Dropdown Items
% Phasing R2	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 3
% Prephasing R1	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 3
% Prephasing R2	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 3
%PF R1	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
%PF R2	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Cluster Density (K/mm^2) R1	Numeric	• Required: No • Read Only: Yes	
Cluster Density (K/mm^2) R2	Numeric	• Required: No • Read Only: Yes	
Intensity Cycle 1 R1	Numeric	• Required: No • Read Only: Yes	
Intensity Cycle 1 R2	Numeric	• Required: No • Read Only: Yes	
Reads PF (M) R1	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Reads PF (M) R2	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Yield PF (Gb) R1	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Yield PF (Gb) R2	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2

Note the following details:

- Values are aggregated across all lanes. Some values (eg, Yield PF (Gb) R1) are summed while others are averaged.
- The names listed previously are the default global custom field names installed with the NovaSeqDx v1.0 configuration provided in the Illumina Preset Protocols (IPP) Package v2.4 or later.
- All global configuration fields are configured on the Container entity.

- All field names are configurable via the Clarity LIMS Configuration > Custom Fields screen (Global Fields tab).
- If field names are changed in Clarity LIMS, they must also be changed through the run.metricUdfNames properties file in the `application.yml` file for the Sequencer API. All fields are configured to be visible on the Record Details screen for the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.0) step (in the Sample Details table). For more information, refer to the application.yml Properties section of *Components Installed* on page 236.

These global custom fields are configured on the Container entity, visible on each input, and must not be confused with per-lane metrics.

All run metrics tracked in Clarity LIMS are overall metrics for the run. There are currently no per-lane metrics provided by NovaSeqDx Operating Software.

Sample Sheet Generation

The sample sheet is generated on the step before the run. This step is either Dilute and Denature (NovaSeqDx v1.0) in the NovaSeqDx Standard protocol or Load to Flowcell (NovaSeqDx v1.0) in the NovaSeqDx Xp protocol. This step places samples on the library tube or flow cell that is loaded in the NovaSeqDx instrument.

In the default configuration, the Validate Run Setup and Generate Sample Sheet automation generates one sample sheet file for use with bcl2fastq v2.20 downstream analysis. This file is in the comma-separated values (CSV) format.

The sample sheet is uploaded to the NovaSeq 6000Dx Operating Software (NVOS) via the `/Illumina/Sequencer/v2/sequencing-run/files` endpoint to the Sequencer API. The file endpoint allows for a file to be downloaded from Clarity LIMS using OAuth (instead of Basic Authentication), which is required for NovaSeqDx.

The run recipe response sets the sample sheet URL to the link to download the file from this endpoint and sets the sampleSheetRequiresOAuth value to true.

Sample Sheet Content

The following step custom fields that display in the Step Details area of the Record Details screen control the sample sheet content:

- Experiment Name
- Read 1 Cycles
- Read 2 Cycles
- Index 1 Cycles
- Index 2 Cycles
- Analysis Software Version

- Override Cycles

For detailed information on the following items, refer to the bcl2fastq2 *Sample Sheet Generation* section of *Illumina Instrument Sample Sheets (NGS v5.17 & later)* on page 2542

- Sample sheet generation script parameters and usage
- Sample sheet data and configuration options
- Enabling unique FASTQ file names per sequencing run

NovaSeq 6000Dx Run

The following steps outline the sequence of events that occurs when a flow cell is loaded onto the NovaSeq 6000Dx instrument.

User Authentication and Login

User authentication and login to the NovaSeqDx instrument is achieved with the getSequencerLoginURL endpoint (`/Illumina/Sequencer/v2/sequencing-run/login`). This endpoint returns the URL that the instrument uses to get an access token.

1. The Sequencer API service provides the NovaSeq 6000Dx Operating Software (NVOS) with a URL for the Clarity LIMS login screen. The NVOS displays this screen to the user. Log in to authorize NVOS to use your account to access Clarity LIMS. This authorization is automatic.
2. Enter credentials:
   - Username and password are passed to the Clarity LIMS server to make sure that the access is valid.
   - By default, the Sequencer API service tries to contact Clarity LIMS at `http://localhost:9080/clarity/`. The installation script sets the URL, but it can be configured with the clarity.url property in the `application.yml` file. For more information, refer to the *application.yml Properties* section of *Components Installed* on page 236.
3. The user is redirected to the Sequencer login page at `/Illumina/Sequencer/v2/sequencer_login`.
   - If authentication is successful, the screen displays a success message. The Clarity LIMS server provides the OAuth token and username by redirecting to `/Illumina/Sequencer/v2/sequencer_login/Success`. This redirect does not reload the page, but allows the NovaSeqDx to continue with run setup.
   - If authentication fails, the login screen does not change and a warning message displays. A background redirect to `/Illumina/Sequencer/v2/sequencer_login/Error` informs the NovaSeq 6000Dx of the failure.

Respond to Validation / Recipe Request Call from Instrument

1. The sequencing-run/recipe/NovaSeq 6000Dx endpoint consumes a POST request with a LibraryContainerId, FlowCellId, and reagents information.
   - LibraryContainerId, FlowCellId, and reagents are provided by the NovaSeq 6000Dx.
   - LibraryContainerId and FlowCellId can match either the library tube ID or flow cell ID of the corresponding containers in Clarity LIMS.
   - If there are no containers or multiple containers, an error is generated.
2. All populated containers whose name matches either the LibraryContainerId or FlowCellId are validated as follows.
   - All samples in the container must be queued for a sequencing run step.
   - All samples must be queued for only one sequencing run step.
   - All samples must be queued for the same sequencing run step.

   Configure the steps that are considered sequencing run steps in the novaseq.sequenceStepNames property in the `application.yml` file. The property is a list and each entry must be the exact name of a sequencing step, not the name of the underlying master step/process type. For more information, refer to the *application.yml Properties* section of *Components Installed* on page 236.
3. After a single container has passed validation, the run setup information (run recipe request) is returned to the NVOS via the API. Then, the run receipt is generated.
   Note the following details:
   - The run recipe information is read from fields configured on the parent process/master step of the samples in the container. The workflow must include recipe definition in that step.
   - By default, the parent process/master step is the Dilute and Denature (NovaSeqDx v1.0) or Load to Flowcell (NovaSeqDx v1.0) step, depending on workflow.
   - The default field values match the names of the fields in the NovaSeq 6000Dx v1.0 configuration included in IPP. The field names are configurable through the recipe.udfNames.* properties. Each property corresponds to an entry in the recipe. The value of a property defines the name of the field that is used to populate that entry in the recipe.
   - It is assumed that this parent process/master step removes any illegal characters from the Experiment Name field.
   - The Clarity LIMS download link to the sample sheet created on the run setup step is used for the SampleSheet value in the recipe. The name of the output of the sample sheet on the setup step is configured through the recipe.sampleSheet.outputName property (Sample Sheet by default) in the `application.yml` file. If no matching output is found, or no file was attached to the

output, the value of the recipe.sampleSheet.notAvailableValue property (Not Available by default) is used in the recipe. For more information, refer to the *application.yml Properties* section of *Components Installed* on page 236.

4. After the recipe is generated, it is returned as a *.json file in the response, in the form defined by the Swagger definition for the API.

Run Recipe Contents

The sample sheet contents and the fields configured on the Dilute and Denature (NovaSeqDx v1.0) and Load to Flowcell (NovaSeqDx v1.0) steps control the contents of the run recipe.

Example of run recipe content is as follows.

```
{
"run_name": "NVOS16027_SPrime_2x250",
"run_mode": "S4",
"workflow_type": "DualIndex",
"librarytube_id": "NV0025867-LIB",
"flowcell_id": "",
"sample_loading_type": "NovaSeqStandard",
"rehyb": false,
"paired_end": true,
"read1": 151,
"read2": 151,
"index_read1": 8,
"index_read2": 8,
"output_folder": "\\\\network_path\\run_data",
"samplesheet": "<path to sample sheet on LIMS server>",
"require_samplesheet_authentication": true,
"usecustomrecipe": false,
"customRecipe": null,
"use_basespace": false,
"basespace_mode": null,
"use_custom_read1_primer": false,
"use_custom_read2_primer": false,
"use_custom_index_read1_primer": false
}
```

Respond to Sequencing Started Call from Instrument

1. Matching container for the run (library tube or flow cell) is found, and contents are validated to be queued for the correct step.

> If no matching container is found, or if the samples in the container are incorrectly queued, an error is thrown and the step is not started. This validation is also done during the run recipe request immediately prior, so this error case should not be encountered.

2. Reagent kits that are configured for the Sequencer API are checked against the configuration for the step to be run:
   - If a kit is configured for the API but does not exist in Clarity LIMS, it is created.
   - If a kit exists, but is not enabled on the step to be run, the step configuration is updated to enable it.
     - If a kit that is to be enabled is Archived, it is set to Active.
     - If a kit that is to be enabled is in Pending status, it is set to Active.
   - If any kits enabled on the step are not configured for the Sequencer API, a warning is logged, and the API cannot complete the step without user intervention.
   - If any kits provided in the recipe request are not configured for the Sequencer API, they are ignored, and a warning is logged during lot tracking.
3. The type of flow cell being used in the run determines the number of replicates for the step.
   - For Standard mode, this information is based on the number of lanes in the flow cell. This information is stored as part of the Sequencer API configuration.
   - For XP mode, there is always one replicate, because there is already one input tracked for each lane.
4. The automated sequencing step is started.
5. Reagent lots are attached to the step. The Flow Cell and Library Tube reagents in the request are ignored. Information from these reagents is tracked in Clarity LIMS as containers and custom fields. Any other reagents in the request are handled as follows.
   - If the reagent is not configured to be tracked, a warning is logged, and the reagent is ignored.
   - You can look up existing reagent lots by kit name (determined by Sequencer API configuration), name (the serial number in the request), and lot number (the lot number in the request).
   - If one or more matching reagent lots exist in Clarity LIMS, the newest one is used.
   - If the expiry date does not match the expiry in the request, or if the status is not set to Active, the lot is updated to have the correct expiry date and an Active status.

   Otherwise a new lot is created with the following configuration:
   - Reagent Kit—Matching kit as determined from Sequencer API configuration and step 2.
   - Name—Serial number in request.
   - Lot Number—Lot number in request.
   - Expiry—Expiry date in request.
   - Status—Active.

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If any reagents are expected to be in the request but are not, these reagents are logged. This action prevents the step from being completed without user intervention.

6. Master step custom fields are updated with the run information parsed from the request. For more information, refer to *Respond to Record InterOps Metrics Call from Instrument* on page 235.
7. Any important events during this request are logged into the Sequencer API log file and in the Sequencing Log multiline global custom field on the step (assuming the step was started successfully). For more information, refer to *Respond to Record InterOps Metrics Call from Instrument* on page 235.

Respond to Record InterOps Metrics Call from Instrument

When the metrics endpoint receives a POST, it completes the following actions:

- Looks for a matching library tube or flow cell in Clarity LIMS.
- Finds the in-progress sequencing step for that container.

When the step is found, the run metrics in the request are written to container global custom fields in Clarity LIMS.

Respond to Sequencing Completed Call from Instrument

If the sequencing run is successful, after the metrics are recorded, the API call for Sequencing Completed is received. The Sequencing Container matches the Information, and the following occurs in Clarity LIMS:

1. The Current Cycle, Current Read, and Status master step custom fields are updated.
2. The multiline Sequencing Log master step field is updated.
3. A success message is recorded in the log file at the info level:

```
Sequencing run with Run ID {runId} of Step '{step.name}' ({step.limsid})
completed successfully.
```

4. Clarity LIMS waits for any automations that are triggered automatically as it advances the step.
5. By default, Clarity LIMS assigns the next steps on the outputs to move them on to the first configured next step. If there are no next steps, the protocol is completed.

Respond to Sequencing Errored Call from Instrument

If the sequencing run is unsuccessful, the API call for Sequencing Error is received. The Sequencing Container matches the Information, and the following occurs in Clarity LIMS:

1. The Current Cycle, Current Read, and Status master step custom fields are updated.
2. The multiline Sequencing Log master step field is updated.
3. A failure message is recorded in the log file at the error level:

```
Step '${Step.name}' (${Step.limsid}) failed with Run ID ${runId}.
```

4. The step is left at the Record Details screen.

Respond to Sequencing RunEndedByUser Call from Sequencer

If the user ends a sequencing run, the API call for Sequencing RunEndedByUser is received. The Sequencing Container matches the Information, and the following occurs in Clarity LIMS:

1. The Current Cycle, Current Read, and Status master fields are updated.
2. The multiline Sequencing Log master step field is updated.
3. The following message is recorded in the log file, at the warning level:

```
"Sequencing run with Run ID ${status.runInfo.runId} of Step
'${step.configuration.name}' (${step.limsid}) aborted by user
${status.runInfo.userName}."
```

4. The step is left at the Record Details screen.

Components Installed

The following sections describe the components (files, properties, reagent categories / label groups, reagent kits, and containers) that are installed by default as part of this integration.

Illumina NovaSeq 6000Dx Integration v1.0 is distributed as an RPM package, BaseSpaceLIMS-sequencer-api. This RPM package must be installed on the Clarity LIMS server.

The BaseSpaceLIMS-sequencer-api RPM installs the following items:

- Sequencer API WAR file
- `application.yml` configuration file
- The following configuration scripts:
  - `configure_sequencer_api_proxy.sh`
  - `configure_sequencer_api_env.sh`
  - `configure_sequencer_api_application.sh`

The NovaSeq 6000Dx v1.0 configuration is delivered through the Illumina Preset Protocols (IPP) Package v2.4. IPP Package v2.4 requires installation of NGS Extensions Package v5.22.0 or later.

- If the NGS Extensions Package is not already installed, or if a version earlier than v5.22.0 is installed, the latest version is installed by default with the NovaSeq 6000Dx integration. For details, refer to *NovaSeq 6000Dx Integration v1.0 Release Notes* on page 264.
- If NGS Extensions Package v5.22.0 or later is already installed, upgrade is not forced or required.

NovaSeq 6000Dx Integration RPM Components

The following table lists the components installed by the RPM package.

Files Installed	Location and Description
`Illumina#Sequencer#v2.war`	`/opt/gls/clarity/tomcat/current/webapps` War file for NovaSeqDx v1.0 API.
`configure_sequencer_api_application.sh`	`/opt/gls/clarity/config/` Script that configures the Sequencer API application through its external `application.yml` file.
`configure_sequencer_api_env.sh`	`/opt/gls/clarity/config/` Script that configures the Clarity LIMS Tomcat configuration to include Secret Util settings.
`configure_sequencer_api_proxy.sh`	`/opt/gls/clarity/config/` Script that configures the proxy to allow communication with the Sequencer API (NovaSeqDx v1.0 API) application.
`sequencer-api.conf`	`/etc/httpd/clarity/` Proxy setting to communicate with Clarity LIMS.

`application.yml` Properties

The `application.yml` file is at `opt/gls/clarity/extensions/sequencing-api/`.

- All properties are configured automatically during installation. You can also configure properties after installation, by editing the `application.yml` file. If you make changes to the file, you must restart Tomcat.
- File properties represent all custom field names, both for recipe and run information.
- For all custom fields, the names (values in this file) can be changed. However, the property names cannot be changed, removed, or added to. For example, the Loading Workflow Type can be changed, but recipe.udfNames.sampleLoadingType cannot.

Table 96 `application.yml` Properties Installed

Property	Description
spring.profiles.active	Tells the application that it is running deployed in Tomcat. Default value: tomcat (Do not change)
clarity.url	Base URL that the Sequencer API service uses to contact the Clarity LIMS. The installation script (configure_sequencer_api_application) prompts for this property.

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Property	Description
clarity.username	Username to be used when communicating with Clarity LIMS.
security.signing-key	Private key that is used when signing/validating OAuth tokens. Changing this property invalidates any issued tokens.
security.token-expiry	Specifies (in hours) the expiry period for login tokens issued by the Sequencer API. Default value: 88
novaseq.sequenceStepNames	List of NovaSeq 6000Dx sequencing run step names for which the integration can find samples queued. • Names must be an exact match to the name of the step in Clarity LIMS (not the master step/process type). • Each line is indented to the same point and start with a dash (-). Default value: • AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.0)

novaseq.flowcells	List of supported NovaSeq 6000Dx flow cell types.
	• Type names exactly match the values used by the NVOS run status requests.
	• To add more types, provide both type and lanes properties, indented to the same point and starting with a dash (-).
	Default value:
	`- type: "SP"`
	`lanes: 2`
	`- type: "S1"`
	`lanes: 2`
	`- type: "S2"`
	`lanes: 2`
	`- type: "S4`
	`"lanes: 4`

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Property	Description

novaseq.reagents	List of supported reagent kits to be tracked on sequencing steps.
	- lotName: The name of the lot as it appears in the run started request.
	- kitName: The name of the corresponding reagent kit in Clarity LIMS that is used for lot tracking.
	• The name can differ in the NVOS request versus the Clarity LIMS configuration.
	Default value:
	`- lotName: "Buffer`
	`"kitName: "Buffer Cartridge"`
	`- lotName: "Cluster"`
	`kitName: "Cluster Cartridge"`
	`- lotName: "SBS"`
	`kitName: "SBS Cartridge"`

recipe.udfNames	Configures the names of the fields used in the run recipe. For more information, refer to *Run Recipe Contents* section in *NovaSeq 6000Dx Run* on page 231. • Each value must be enclosed in quotes. Example: recipe.udfNames.runMode "Run Mode" • Value provided for sampleLoadingType must match the value provided in the run configuration. Example: recipe.udfNames.sampleLoadingType "Loading Workflow Type"
recipe.sampleSheet.outputName recipe.sampleSheet.notAvailableValue	Configures the name of the sample sheet file placeholder on the step where run recipe information is populated, and the property value to use when the file is not found.

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Property	Description
run.stepUdfNames	Configures the master step field names to be used when recording run results. For more information, refer to the *Master Step Fields* section in *NovaSeq Run* on page 583 • Each value must be enclosed in quotes. Example: run.stepUdfNames.runID "Run ID" • Value provided for sampleLoadingType must match the value provided in the run configuration. • Example: run.stepUdfNames.sampleLoadingType "Loading Workflow Type". It is expected that the run step field and the field on the input derived sample share a name.
run.metricUdfNames	Configures the result file global custom field names to be used when recording run results. These are the fields that capture the run data. For more information, refer to *Global Custom Fields* section.
run.autoComplete	Determines whether the sequencing run step is completed automatically after parsing the run metrics on a run (refer to run.autoCompleteOnlyAtSuccess). Default value: true For more information, refer to the *Configure Autocomplete Properties* on page 242 section.
run.autoCompleteOnlyAtSuccess	Determines which of the following options are applicable to the sequencing run step: • Autocompleted when the run status is RunCompletedSuccessfully (if autoCompleteOnlyAtSuccess is true) • Autocompleted regardless of the run status (if autoCompleteOnlyAtSuccess is false). Default value: true For more information, refer to the *Configure Autocomplete Properties* on page 242 section.

Reagent Categories/Label Groups

- TruSeq HT Adapters v2 (D7-D5)

Reagent Kits

- Buffer Cartridge

- Cluster Cartridge
- DPX1
- DPX2
- DPX3
- NaOH
- Resuspension Buffer (RSB)
- SBS Cartridge
- Tris-HCl

Container Types

- Library Tube
- SP
- S1
- S2
- S4

This integration supports the following items:

- Library tube with barcode provided in the following formats:
  - RUO mode: [A-Z]{2}[0-9]{7}-[A-Z]{3}
    Example: AB1234567-LIB
  - DX mode: [A-Z]{2}[0-9]{7}-[A-Z]{5}
    Example: AB1234567-LIBDX
- SP, S1, S2, or S4 flow cell with barcode provided in one of the following formats:
  - SP and S1 flow cell: [A-Z0-9]{5}DRX[XY2357]
  - S2 flow cell: [A-Z0-9]{5}DMX[XY2357]
  - S4 flow cell: [A-Z0-9]{5}DSX[XY2357]
    Example: H1991DMXX

NVOS Configuration

For details on configuring the NovaSeq 6000Dx Operating Software (NVOS) for integration with Clarity LIMS, contact the Clarity LIMS Support team.

Routing Script Requirements

The requirements for the routing script functionality are as follows.

- On the steps that use the routing script (Define Run Format (NovaSeqDx v1.0) and Dilute and Denature (NovaSeqDx v1.0)):

  - The Next Step for all samples must be set to Remove from workflow. A script sets this value. The value must not change in the Assign Next Steps screen.
- In the protocol configuration screen, the following settings are required and must not be changed:
  - In the Next Steps section, for the last step in the protocol, the method of assigning the next step must be set to Automatic.

Configure Autocomplete Properties

The `application.yml` configuration file contains l the following properties:

- run.autoCompleteOnlyAtSuccess
- run.autoComplete

These properties determine the conditions under which the last step of the NovaSeq 6000Dx workflow, AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.0), is automatically completed. The two scenarios for autocompletion of the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.0) step are as follows.

- The step autocompletes only when the run status is RunCompletedSuccesfully (default behavior).
- The step autocompletes regardless of the run status.

By default, both properties are set to true and the step only completes if the run is successful. If the run fails or is aborted, the step must be completed manually in Clarity LIMS.

For the step to autocomplete regardless of the result of the sequencing run, change the autoCompleteOnlyAtSuccess property value to false.

Enable/Disable autoComplete Properties

1. Open the `application.yml` file at `/opt/gls/clarity/extensions/sequencer-api/application.yml`:

```
vi /opt/gls/clarity/extensions/sequencer-api/application.yml
```

2. Edit the value of the autoCompleteOnlyAtSuccess property as required.
3. Save the file.

autoComplete Properties

The following table shows how the combined value of the run.autoComplete and run.autoCompleteOnlyAtSuccess properties affects the autoComplete behavior of the sequencing step.

Table 97 run.autoComplete and run.autoCompleteOnlyAtSuccess Value Matrix

run.autoComplete property value	run.autoCompleteOnlyAtSuccess property value	Outcome
true	true	• Step automatically completes only if the sequencing run is successful. • If run is not successful, the step does not automatically complete, and the run details are recorded in Clarity LIMS.
true	false	• Step automatically completes, regardless of the run status.
false	true	• Step does not automatically complete. • Run details are recorded in Clarity LIMS.
false	false	

Rules and Constraints

- The workflow configuration contains several validation checks. To make sure that the calculations work properly, it is important that you do not disable any of this validation logic. The validation checks determine the following information:
  - Which samples, and how many, can enter each step together.
  - Which samples, and how many, can be pooled together.
- The library tube ID must be unique. There must not be multiple library tube containers in the system with the same name.
- Reagent labels (indexes) must be unique.
- Only controls are permitted as unindexed samples. All other unindexed samples and pools are not permitted.
- For sample sheet generation constraints, refer to the *Bcl2fastq2 Sample Sheet Generation* section in *Illumina Instrument Sample Sheets (NGS v5.17 & later)* on page 2542.
- Do not manually start the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.0) step. This step is fully automated and the sequencing service does not update samples correctly if they have been manually started.
- For the automated run to start successfully, the Validate Run Setup and Generate Sample Sheet button must be selected.

NovaSeq 6000Dx Integration v1.0 Validation and Troubleshooting

This section explains how to validate the installation of the Illumina NovaSeq 6000Dx Integration Package v1.0.

The validation process involves the following items:

- Running samples through the Library Prep Validation workflow.
  - The workflow contains a single-step protocol that models the library prep required to produce normalized libraries. At the end of the step, the normalized libraries advance to the workflow selected.
- Running normalized libraries through the NovaSeqDx v1.0 workflow validates the following items:
  - Successful routing of samples from the Run Format (NovaSeqDx v1.0) step to the NovaSeqDx Standard (NovaSeqDx v1.0) or NovaSeqDx Xp (NovaSeqDx v1.0) step.
  - Automated generation of v2 sample sheet for use with bcl2fastq2 v2.20 analysis software. This file automatically uploads to the sequencing system via the Sequencer API.
  - Automatic validation of run setup information. Information is uploaded to the NovaSeq 6000Dx Operating Software (NVOS) via the Sequencer API and is used to create the run recipe and initiate the run.
  - Automated tracking of the NovaSeq 6000Dx sequencing run and parsing of run statistics into Clarity LIMS, via the Sequencer API.

The validation steps assume that the NovaSeq 6000Dx Integration Package v1.0 is installed and you have imported the default Clarity LIMS configuration.

Activate Workflow, Create Project, Add and Assign Samples

The following steps set up Clarity LIMS in preparation for running samples through the Library Prep Validation and NovaSeq 6000Dx v1.0 workflows.

1. On the Configuration tab, under Workflows, activate both the Library Prep Validation v2.2.0 and NovaSeq 6000Dx v1.0 workflows.
2. On the Projects and Samples screen, create a project and add samples to it.
3. Assign the samples to the Library Prep Validation workflow.

Library Prep Protocol: Library Prep Validation v2.2.0

This single-step protocol models the library prep required to produce normalized libraries that are ready for the NovaSeq 6000Dx v1.0 workflow.

Run Library Prep Validation v2.2.0

1. In Lab View, locate the Library Prep Validation v2.2.0 protocol.
   Samples are queued for the Library Prep Validation v2.2.0 step.

2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Placement screen, complete the following actions:
   a. In the Placed Samples area on the right, in the Container field, scan or enter the barcode of the destination container.
   b. Select the samples from the Samples to be Placed area on the left, and drag them to the container wells on the right.
   c. Select Add Labels.
5. On the Add Labels screen, the Labels to Add list is populated with labels from the TruSeq HT Adapters v2 (D7-D5) label group. The group is the only label group configured in the integration and is used by default in this step.
   a. Select labels on the left, and drag them to the container on the right to assign them to placed samples.
   b. Select Record Details.
6. On the Record Details screen, complete the following actions:
   a. In the Sample Details table, enter values for Normalized Molarity. Type values individually or copy and paste to enter multiple values from a spreadsheet.
   b. The Sequencing Instrument column indicates the instrument to be used for the sequencing run. Select NovaSeqDx.
   c. Populate the fields in this column using one of the following methods:
      - To populate fields individually for each sample, select from the drop-down list in each row.
      - To populate all fields at one time, copy and paste from a spreadsheet.

   Alternatively, select inside the table and press Ctrl + A to select all rows. At the top of the table, select Sequencing Instrument and then select NovaSeqDx from the adjacent drop-down list. Select Apply.

   If working with many samples, copy and paste to insert multiple values from a spreadsheet as follows.

   a. Select Copy Samples to copy the sample information from Clarity LIMS to the Clipboard.
   b. Open a blank Excel document and paste the contents of the clipboard into the spreadsheet.
   c. In the spreadsheet, enter the Normalized Molarity and Sequencing Instrument values. Select the entire spreadsheet including the headers (Ctrl + A), and copy (Ctrl + C) the information to the Clipboard.
   d. Within Clarity LIMS, select the Paste Samples button. Paste the contents of the clipboard (Ctrl + V) into the Update Table window and then select Update to paste the contents into the Sample Details table.
7. Select Next Steps.

Triggers the Next Steps automation, which sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step.

8. On the Assign Next Steps screen in the Samples table, the Next Step value for all samples is prepopulated with Remove from workflow.

   Do not change this value. If Next Step is not set to Remove from workflow, the routing script does not route samples correctly.

9. Select Finish Step.
   On exit from the step, the Routing Script automation is triggered. This automation assigns samples to the first step of the NovaSeq 6000Dx v1.0 workflow, Define Run Format (NovaSeqDx v1.0). This is the only step in Protocol 1: Run Format (NovaSeqDx v1.0).

Protocol 1: Run Format (NovaSeqDx v1.0)

This protocol includes a single step, Define Run Format (NovaSeqDx v1.0). The step allows for the assignment of per sample values for the following fields:

- NovaSeqDx Run Mode
- Loading Workflow Type
- Normalized Molarity
- Flowcell Type
- Final Loading Concentration (pM)

At the end of the step, samples are routed to the NovaSeqDx Standard or NovaSeqDx Xp protocol. This routing is done according to the selected Loading Workflow Type. The selected NovaSeqDx Run Mode must be compatible with the Loading Workflow Type and Flowcell Type.

Step 1: Run Define Run Format (NovaSeqDx v1.0)

1. In Lab View, locate the Run Format (NovaSeqDx v1.0) protocol. The samples are queued for the Define Run Format (NovaSeqDx v1.0) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Record Details screen in the Sample Details table, populate the following fields (values can vary across samples):
   - NovaSeqDx Run Mode—Select DX or RUO.
   - Loading Workflow Type—Select NovaSeq Standard or NovaSeq Xp from the drop-down list.
   - Flowcell Type—Select SP, S1, S2, or S4.
   - Final Loading Concentration (pM)—Select from the two preset options: 225 (for PCR-free workflows), 400 (for Nano workflows), or enter a different value.

- Normalized Molarity (nM)—These values are copied over from the previous step. If this column is not populated during library prep, enter the values here.

5. Select Next Steps, which triggers the Next Steps automation and the following actions:
   - Sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step.
   - Checks that the NovaSeqDx Run Mode value is compatible with the Loading Workflow Type and Flowcell Type. This automation also generates an error message if the Loading Workflow Type or the Flowcell Type are incompatible.
   - Calculates the Minimum Molarity.
   - Checks Normalized Molarity value. For samples with no Normalized Molarity value (eg, empty value, not including 0), generates an error message notifying that the field cannot be empty.
   - Compares each sample Normalized Molarity value with the Minimum Molarity value.
6. On the Assign Next Steps screen:
   - In the Sample Details table, the Next Step for all samples is prepopulated with Remove from workflow, regardless of the Loading Workflow Type.

     Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not routed samples correctly.

   - For samples whose Normalized Molarity value is lower than the Minimum Molarity value, the Loading Workflow Type is set to Remove from workflow. A message is recorded in the Warning field for the sample.
     To proceed, choose one of the following options:
     - Return to the Record Details screen and adjust the Normalized Molarity value so that it equals or exceeds the Minimum Molarity value. The Loading Workflow Type must be set to NovaSeq Standard or NovaSeq Xp, as applicable.
     - Complete the protocol without correcting the Normalized Molarity value. In this case, the samples in question are removed from the Clarity LIMS workflow.
7. Select Finish Step. The Routing Script automation is triggered:
   - Samples whose Loading Workflow Type is set to Remove from workflow are removed from the Clarity LIMS workflow. (Occurs when the Normalized Molarity value is lower than the Minimum Molarity.)
   - Samples whose Loading Workflow Type is set to NovaSeq Standard are routed to the Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.0) step. This step is the first step in the NovaSeq Standard protocol.
   - Samples whose Loading Workflow Type is set to NovaSeq Xp are routed to the Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.0) step. This step is the first step in the NovaSeq Xp protocol.

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Protocol 2: NovaSeqDx Standard (NovaSeqDx v1.0)

In this protocol, samples are pooled and added to the library tube in preparation for the NovaSeq 6000Dx run. The protocol contains two steps:

1. Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.0)
2. Dilute and Denature (NovaSeqDx v1.0)

Step 1: Run Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.0)

1. In Lab View, locate the NovaSeqDx XP (NovaSeqDx v1.0) protocol.
   Samples for the Make Bulk Pool for NovaSeqDx Standard (NovaSeqDx v1.0) step are queued.
2. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
   When the step begins, the Validate Single NovaSeqDx Run Mode automation is triggered. This automation checks that the samples have the same NovaSeqDx Run Mode (RUO or DX).
4. On the Pooling screen, perform the following actions:
   a. Create a pool by dragging samples into the Pool Creator.

      Only create one pool.

   b. Enter a name for the pool or accept the default name (eg, Pool #1).
   c. Select Record Details.
      After exiting the Pooling screen, the Validate Inputs Flowcell Type and Single Pool is triggered. The automation verifies the following items:
      - All samples in the pool have been assigned the same Flowcell Type.
      - Only one pool has been created.
5. On the Record Details screen in the Step Details area, complete the two required fields and one optional field, as needed:
   - Number of Flowcells to Sequence—Used in volume calculations, to make sure that the volumes are sufficient for the number of times the pool is sequenced.
   - Minimum Per Sample Volume (µl)—Used to calculate how much of each sample is included in the pool. The field is prepopulated with the configured default value 5 µl, but can be edited.
   - % PhiX (2.5 nM) Spike-In—Used to calculate the volume of PhiX v3 control included in the pool for the given percentage of spike-in. The field is optional and can be edited.

Assuming the default Minimum Per Sample Volume (µl) value of 5, for a given batch:

- If the smallest Per Sample Volume (µl) value is < 5, Clarity LIMS automatically assigns a value of 5 to the Adjusted Per Sample Volume (µl) field.
- Clarity LIMS changes the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

6. In the Sample Details table, select the pool icon to view details on the pool composition.
7. Select Calculate Volumes to trigger the Calculate Volumes automation. This automation performs calculations based on the selected Flowcell Type.
   - Calculates Bulk Pool Volume (µl) value.
   - Sets the value of the Bulk Pool Volume (µl) field.
   - Calculates the PhiX Volume (µl) value if % PhiX (2.5 nM) Spike-In has value.
   - Calculates the Per Sample Volume (µl) value to be added to the pool.
   - Calculates the Total Sample Volume (µl) value.
   - If the Total Sample Volume is less than the Bulk Pool Volume, it calculates the RSB Volume (µl) value.
   - Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
   - Populates the Volume of Pool to Denature (µl), NaOH Volume (µl) and Tris-HCl Volume (µl) columns of the Sample Details table. The script sets the values, and is not editable.
   - The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains volume information about the pool and the individual samples that it contains. Select the file to download it and then open it in Excel.
8. Select Next Steps to trigger the Set Next Step automation. This automation sets the next step for samples to ADVANCE and advances them to the next step in the protocol, Dilute, and Denature (NovaSeqDx v1.0).
   On the Assign Next Steps screen, the next step for samples is set to the next step in the workflow: Dilute and Denature (NovaSeqDx v1.0).
9. Select Finish Step.
   At the end of this step, the pool of samples automatically advances to the Dilute and Denature (NovaSeqDx v1.0) step.

Step 2: Run Dilute and Denature (NovaSeqDx v1.0)

1. In Lab View, locate the NovaSeqDx Standard (NovaSeqDx v1.0) protocol. The pool of samples queued for the Dilute and Denature (NovaSeqDx v1.0) step are listed.
2. Add the samples to the Ice Bucket and select View Ice Bucket.

3. On the Ice Bucket screen, select Begin Work. The Validate Single Input automation is triggered. This automation verifies that only one container is input for the step.
4. On the Placement screen:
   a. Drag the pool into the library tube in the Placed Samples area.
   b. Scan or type the barcode of the library tube into the Library Tube field.
   c. Select Record Details.

   After exiting the Placement screen, the Validate Library Tube Barcode automation makes sure that the library tube barcode conforms to the barcode mask that corresponds with the NovaSeqDx Run Mode in the following table. If the barcode does not match, an error message displays. The automation also copies the Flowcell Type and Loading Workflow Type values from step inputs to outputs.

NovaSeqDx Run Mode	Valid Barcode Mask
RUO	[A-Z]{2}[0-9]{7}-[A-Z]{3}
DX	[A-Z]{2}[0-9]{7}-[A-Z]{5}

5. On the Record Details screen, the Reagent Lot Tracking section lets you tracks the NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. Add and activate lots for these reagents as follows.
   a. In a new browser tab, open an additional instance of Clarity LIMS.
   b. Navigate to the Consumables configuration screen.
   c. Select the NaOH reagent kit and select New Lot.
   d. Enter the lot details.
   e. Set the Status of Reagent Lot toggle switch to Active.
   f. Select Save.
   g. Repeat this process to add and activate Resuspension Buffer and Tris-HCl reagent lots.
   h. Return to your original working browser tab and refresh the page.
6. In the Reagent Lot Tracking section, select from the active lots displayed in each drop-down list. The fields displayed on the Record Details screen are used to set up the run and generate the sample sheet. Some of these fields are autopopulated and some must be completed manually.

Table 98 Fields Displayed on Record Details Screen of Denature and Dilute (NovaSeqDx v1.0)

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.

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Field	Value
BaseSpace Sequence Hub Configuration	Select from the following preset options: • Not Used • Run Monitoring Only • Run Monitoring and Storage
Workflow	Automatically populated with the preset GenerateFASTQ.
Workflow Type	Select from the following preset options: • No Index • Single Index • Dual Index • Custom
Index Read 1	Select from the following preset options: • 0 • 6 • 8 • type a value between 0 and 20
Index Read 2	Select from the following preset options: • 0 • 6 • 8 • type a value between 0 and 20
Paired End	Select from the following preset options: • True • False
Read 1 Cycles	Select from the following preset options: • 251 • 151 • 101 • 51 • type a value between 1 and 251 *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.

Field	Value
Read 2 Cycles	Select from the following preset options: • 251 • 151 • 101 • 51 • type a value between 0 and 251 *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Use Custom Read 1 Primer	Select if applicable.
Use Custom Read 2 Primer	Select if applicable.
Use Custom Index Read 1 Primer	Select if applicable.
Use Custom Recipe	Select if applicable.
Custom Recipe Path	If the Use Custom Recipe option is selected, enter the path to the custom recipe file to be used.
Output Folder	Enter network path for sequencing run folder. For example: `\\networkshare\run_data`
Analysis Software Version	Enter the software version for BCL Convert Analysis for v2 sample sheet. Leave blank if not applicable.
Override Cycles	Enter the Override Cycles value for v2 sample sheet. Leave blank if not applicable.

7. On the Record Details screen, select Validate Run Setup and Generate Sample Sheet. This triggers the automation script, which performs the following actions:
   - Validates the parameters entered on the Record Details screen.
   - Generates the sample sheet and attaches it to the placeholder in the Files area of the Record Details screen.

     The v2 sample sheet generated does not include Cloud and Analysis sections. Contact Illumina Support for information on generating v2 sample sheets with those sections.

   - Select Next Steps.
8. On the Assign Next Steps screen, the Next Step for samples is prepopulated with Remove from workflow. The Routing Script automation expects this value, and requires the value to advance samples to the next step.

> Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.

9. Select Finish Step.
   After exiting the step, the following actions occur:
   - The Routing Script automation is triggered and samples are routed to AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.0).
   - In Lab View, the pool of samples is queued for the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.0) step.

   At this point in the workflow, the user interaction ends. Proceed to AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.0).

   > Do not add samples to the Ice Bucket or start the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.0) step. The integration adds samples automatically.

Protocol 3: NovaSeqDx Xp (NovaSeqDx v1.0)

In this protocol, samples are pooled and added to lanes on the NovaSeq 6000Dx flow cell. The option selected in the Define Run Format (NovaSeqDx v1.0) step determines the flow cell type.

The protocol contains three steps:

1. Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.0)
2. Dilute, Denature & ExAmp (NovaSeqDx v1.0)
3. Load to Flowcell (NovaSeqDx v1.0)

Step 1: Run Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.0)

1. In Lab View, locate the NovaSeqDx Xp (NovaSeqDx v1.0) protocol. The samples are queued for the Make Bulk Pool for NovaSeqDx Xp (NovaSeqDx v1.0) step.
2. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Pooling screen, do as follows.
   a. Create a pool by dragging samples into the Pool Creator.

      > Create only one pool.

   b. Enter a name for the pool or accept the default name (eg, Pool #1).
   c. Select Record Details.
5. On exiting the Pooling screen, the Validate Inputs Flowcell Type and Single Pool automation is triggered. The automation verifies the following items:
   - All samples in the pool are assigned the same Flowcell Type.

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- Only one pool was created.

6. On the Record Details screen, in the Step Details area, complete the two required fields and one optional field, as needed:
   - Number of Lanes to Sequence—Used in volume calculations to make sure that volumes are sufficient for the number of times the pool is sequenced.
   - Minimum Per Sample Volume (µl)—Used to calculate how much of each sample is included in the pool. The field is prepopulated with the configured default value 5 µl, but can be edited.
   - % PhiX (0.25nM) Spike-In—Used to calculate the volume of PhiX v3 control to be included in the pool for the given percentage of spike-in. The field is optional and can be edited.

     Assuming the default Minimum Per Sample Volume (µl) value of 5, for a given batch:
     - If the smallest Per Sample Volume (µl) value is < 5, Clarity LIMS automatically assigns a value of 5 to the samples Adjusted Per Sample Volume (µl) field.
     - Clarity LIMS then adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

7. [Optional] In the Sample Details table, select the pool icon to view details on the pool composition.
8. Select Calculate Volumes to trigger the Calculate Volumes automation. This automation performs volume calculations based on the selected Flowcell Type, as follows.
   - Calculates Bulk Pool Volume (µl) value.
   - Calculates the Per Sample Volume (µl) value to be added to the pool.
   - Calculates PhiX Volume (µl) value if % PhiX (0.25nM) Spike-In has value.
   - Calculates the Total Sample Volume (µl) value.
   - If the Total Sample Volume is less than the Bulk Pool Volume, the automation calculates the RSB Volume (µl) value.
   - Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
   - Generates the Calculation File (CSV) and attaches it to the step. This file contains volume information about the samples and RSB buffer to add to the pool. Select the file to download it, and then open it in Excel.
9. Select Next Steps to trigger the Set Next Step automation. This automation performs the following actions:
   - Copies the Flowcell Type values from the step inputs to the step outputs.
   - Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol—Dilute, Denature & ExAmp (NovaSeqDx v1.0).

   On the Assign Next Steps screen, the next step for samples is set to Dilute, Denature & ExAmp (NovaSeqDx v1.0).

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10. Select Finish Step.
    At the end of this step, the pool of samples automatically advances to the Dilute, Denature & ExAmp (NovaSeqDx v1.0) step.

Step 2: Run Dilute, Denature & ExAmp (NovaSeqDx v1.0)

1. In Lab View, locate the NovaSeqDx Xp (NovaSeqDx v1.0) protocol. The pool of samples queued for the Dilute, Denature & ExAmp (NovaSeqDx v1.0) step displays.
2. Add the pool to the Ice Bucket and select View Ice Bucket.
3. [Optional] On the Ice Bucket screen, set the number of derivatives to create (placed into the flow cell lanes) and select Begin Work.
4. On entry to the Record Details screen, the Calculate Volumes automation is triggered.
   This automation sets the following values based on the selected Flowcell Type:
   - BP Aliquot Volume (µl)
   - NaOH Volume (µl)
   - Tris-HCl Volume (µl)
   - DPX1 Volume (µl)
   - DPX2 Volume (µl)
   - DPX3 Volume (µl)
   - Mastermix per Lane (µl)
   - The automation also populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
   - The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains information about the DPX Mastermix volume and the volume of Mastermix, NaOH, and Tris-HCl to add per working pool (refer to the next step).
5. On the Record Details screen, the Reagent Lot Tracking section tracks the DPX1, DPX2, DPX3, NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. These reagents must be added.
   To add and activate reagent lots, do as follows.
   a. In a new browser tab, open an additional instance of Clarity LIMS.
   b. Select one of the reagent kits previously listed and select New Lot.
   c. Enter the lot details.
   d. Set the Status of Reagent Lot toggle switch to Active.
   e. Select Save.
   f. Repeat this process to add and activate the remaining reagent lots.
   g. Return to your original working browser tab and refresh the page.

6. Add and activate reagent lots as follows.
   On the Record Details screen, the Reagent Lot Tracking section tracks the reagents used in the step.
   a. In a new browser tab, open another instance of Clarity LIMS.
   b. Navigate to the Consumables screen.
   c. Select one of the following reagent kits:
      - DPX1
      - DPX2
      - DPX3
      - NaOH
      - Resuspension buffer
      - Tris-HCl
   d. Select New Lot.
   e. Enter the lot details.
   f. Set the Status of Reagent Lot toggle switch to Active.
   g. Select Save.
   h. Repeat steps c–g for the remaining reagent lots.
   i. Return to the original instance of Clarity LIMS and refresh.
7. On the Record Details screen, perform the following actions:
   a. In the Reagent Lot Tracking section, select from the active lots displayed in each drop-down list.
   b. In the Step Details section, verify that the DPX1, DPX2, and DPX3 reagent volume values are populated. The script sets these values and they cannot be edited.
   c. [Optional] In the Sample Details table, select the pool icon to view details of the working pool composition.
   d. Make sure that the following values and columns are populated:
      - BP Aliquot
      - Mastermix per lane
      - NaOH
      - Tris-HCl
      - Flowcell Type
      - Loading Workflow Type

      A script sets these values and columns and they cannot be edited. The working pool number is appended to the bulk pool name, so that you can identify which working pools are derived from the same bulk pool.

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8. In the Files area, select the Calculation File (CSV) to open it and view details on the DPX Mastermix volume and the volume of Mastermix, NaOH, and Tris-HCl to add per working pool.
9. Select Next Steps.
   On the Assign Next Steps screen, the next step is already set to Load to Flowcell (NovaSeqDx v1.0).
10. Select Finish Step.

Step 3: Run Load to Flowcell (NovaSeqDx v1.0)

1. On the Ice Bucket screen, perform the following actions:
   a. In the Container Options panel, select the appropriate flow cell type from the Destination Container drop-down list.
   b. Select Begin Work.
2. The Validate Inputs and Selected Container automation checks the following information:
   - The Flowcell Type field is set to a valid value (SP, S1, S2, or S4) and each input has the same value for the field.
   - The container type selected matches the value in the Flowcell Type field.
   - The number of outputs matches the number of lanes on the selected flow cell type. If validation fails, an error message reports the number of working pools does not match the number of lanes available on the flow cell.
3. On the Placement screen, do as follows.
   a. Drag pools from the left of the screen over into the Placed Samples area on the right.
   b. Scan or type the barcode of the flow cell into the Flowcell field.
   c. Select Record Details.
   After exiting the Placement screen, the Validate Flowcell Barcode automation makes sure that the container barcode conforms to the barcode mask for the selected Flowcell Type. This automation also copies the Flowcell Type and Loading Workflow Type field values from step inputs to outputs. The following table shows the barcode mask information.

Flowcell Type	Barcode Mask
SP	[A-Z0-9]{5}DRX[XY2357]
S1	[A-Z0-9]{5}DRX[XY2357]
S2	[A-Z0-9]{5}DMX[XY2357]
S4	[A-Z0-9]{5}DSX[XY2357]

4. The fields displayed on the Record Details screen are used to set up the run and generate the sample sheet.
   Some of these fields are autopopulated and some must be entered manually. Refer to the following table for details.

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Table 99 Fields Displayed on Record Details Screen of Load to Flowcell (NovaSeqDx v1.0) Step

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. No spaces.
BaseSpace Sequence Hub Configuration	Presets • Not Used • Run Monitoring Only • Run Monitoring and Storage
Workflow	Presets • GenerateFASTQ
Workflow Type	Presets • No Index • Single Index • Dual Index • Custom
Index Read 1	Presets • 0 • 6 • 8 • Type a value between 0 and 20
Index Read 2	Presets • 0 • 6 • 8 • Type a value between 0 and 20
Paired End	Presets • True • False
Read 1 Cycles	Presets • 251* • 151 • 101 • 51 • Type a value between 1 and 251 *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.

Field	Value
Read 2 Cycles	Presets • 251* • 151 • 101 • 51 • Type a value between 0 and 251 *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Use Custom Read 1 Primer	Select if applicable.
Use Custom Read 2 Primer	Select if applicable.
Use Custom Index Read 1 Primer	Select if applicable.
Use Custom Recipe	Select if applicable.
Custom Recipe Path	If you selected the Use Custom Recipe option, enter the path to the custom recipe file to be used.
Output Folder	Enter network path for sequencing run folder. For example: `\\networkshare\run_data`
Library Tube Barcode	Scan the library tube barcode.
Analysis Software Version	Enter the software version for BCL Convert Analysis for v2 sample sheet. Leave blank if not applicable.
Override Cycles	Enter the Override Cycles value for v2 sample sheet. Leave blank if not applicable.

5. Select Validate Run Setup and Generate Sample Sheet, and then select Next Steps to trigger the automation script. The script performs the following actions:
   - Validates the parameters entered on the Record Details screen.
   - Generates the sample sheet and attaches it to the placeholder in the Files area on the Record Details screen.

   The v2 sample sheet generated does not include Cloud and Analysis sections. Contact Illumina Support for information on generating v2 sample sheets with these sections.

6. Select Next Steps.
   On the Assign Next Steps screen, the Next Step field for samples is prepopulated with Mark protocol as complete.
7. Select Finish Step.

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At this point in the workflow, the user interaction ends. The flow cell is queued for the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.0) step.

Proceed to AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.0).

Do not add samples to the Ice Bucket or start the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.0) step. The integration adds samples automatically.

Protocol 4: AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.0)

This protocol contains a single fully automated step - AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.0).

The integration starts the step automatically and data from the run is parsed back into Clarity LIMS. User interaction is not required, but you can review the stages of the step in Clarity LIMS (refer to Review Run Data).

Automations

The NovaSeqDx Run (NovaSeqDx v1.0) step contains two automations:

- Set Next Steps—This automation is disabled (Set to Not Used). By default, the Sequencer API is used to set the next step for output samples.
  The Illumina Preset Protocols (IPP) Package include a Set Next Steps automation configured on the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.0) step. You can use this automation to override the default next step behavior. For more information, refer to *NovaSeq 6000Dx Integration v1.0 Configuration and User Interaction* on page 185.
- Update Lane Number—This automation is triggered automatically upon entry to the Record Details screen. To make sure that the Lane Number displays correctly, do not disable or modify this automation.

Review Run Data

Read summary metrics are recorded for the library pool. After the run is complete, open the step and review these metrics displayed on the Next Steps screen in the Step Data section and the Sample Details table.

Step Data Section

The following values populate the master step fields:

- Run ID
- Run Status
- Output Folder
- Current Cycle

- Current Read
- Loading Workflow Type
- Flow Cell ID
- Flow Cell Side
- Flow Cell Mode
- Flow Cell Part Number
- Flow Cell Lot Number
- Flow Cell Expiration Date
- Instrument ID
- Instrument Type
- Instrument Control Software Version
- Firmware Version
- RTA Version
- Sequencing Log

Sample Details Table

Summary metrics populate the global custom fields listed. Values are aggregated across all lanes. Some values (eg, Yield PF (Gb) R1) are summed, while others are averaged.

- Yield PF (Gb) R1
- Yield PF (Gb) R2
- Reads PF (M) R1
- Reads PF (M) R2
- Cluster Density (K/mm^2) R1
- Cluster Density (K/mm^2) R2
- %PF R1
- %PF R2
- % Bases >=Q30 R1
- % Bases >=Q30 R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- % Phasing R1
- % Phasing R2

- % Prephasing R1
- % Prephasing R2
- % Aligned R1
- % Aligned R2
- % Error Rate R1
- % Error Rate R2

How the Integration Works

The following steps summarize how the Sequencer API integration works. For details on sample sheet generation and the sequence of events involved in the NovaSeq run, refer to *NovaSeq 6000Dx Integration v1.0 Configuration and User Interaction* on page 185.

1. When setting up a run on the NovaSeq 6000Dx instrument, NVOS sends a request for the run recipe.
   a. The Sequencer API validates that samples and containers are correctly queued for the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.0) step.
   b. Clarity LIMS sends a JSON response to NVOS. The response includes the run recipe information and a link to download the sample sheet (used with analysis software such as bcl2fastq2).
2. When the run starts, NVOS sends a RunStarted run status request. The Sequencer API then does the following:
   a. Validates reagent kit information in the RunStarted request:
      - Verifies that the reagent kit exists. If the reagent kit does not exist, the Sequencer API creates the kit and enables on the master step.
      - Verifies that the reagent kit is activated. If the reagent kit is not active, the Sequencer API activates it.
   b. Initiates the step for the queued samples. The step produces one output file placeholder per lane of the flow cell in use, based on the flow cell type.
   c. Records all relevant information from the RunStarted request on the step (such as reagent lots and step fields).
3. At the end of the run, NVOS sends one of the following run status requests:
   - RunCompletedSuccessfully
   - RunEndedByUSer
   - RunErroredOut

   The Sequencer API updates the step with any new information, which includes only the status and cycle/read information.

4. After primary analysis completes, NVOS sends a request containing the parsed run metrics. NVOS sends a request only when the run completes successfully. However, the API does not make this assumption and accepts the request regardless of status. The Sequencer API then does the following:
   a. Records the metrics into the fields on file placeholder outputs in Clarity LIMS.
   b. Completes the step in Clarity LIMS if the status is RunCompletedSuccessfully. For all other status options, the step remains in progress. This action is the default autocomplete step behavior. For more information, refer to *NovaSeq 6000Dx Integration v1.0 Configuration and User Interaction* on page 185.

   > *NovaSeq 6000Dx Integration v1.0 Configuration and User Interaction* on page 185 provides detailed information on how the following components work with the Sequencer API integration:
   >
   > - Sample sheet generation and contents
   > - NovaSeq 6000Dx run
   > - User authentication and login
   > - Run recipe contents

Troubleshooting

If an automation trigger does not appear to run its corresponding scripts, refer to the following sections in the Clarity LIMS API documentation:

- *Troubleshooting Automated Worker*
- *Troubleshooting Automation* in the API

If an error occurs that does not provide direction on how to proceed, do as follows.

1. Confirm the version of the installed Illumina NovaSeq 6000Dx Integration Package by running the following command on the Clarity LIMS server command line:

```
rpm -qa | grep -i novaseq
```

2. If the error is related to the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.0) step, review the log file information. For more details, refer to the *Logging* on page 264 section.

If the automated run step starts, but does not complete, do as follows.

1. Use one of the following methods to open the in progress step in Clarity LIMS:
   - Log in to the default user account.
   - In Lab View, find the step in the Recent Activities pane.
   - Search for the step in Clarity LIMS. You can use the Library Tube or Flowcell barcode as the search term. The steps depend on whether the search is a NovaSeqDx Standard or NovaSeqDx Xp run.
2. On the Record Details screen, the Sequencing Log multiline text field contains logging information.

If unable to reach the Record Details screen, or if the Sequencing Log field does not contain enough information to resolve the issue, review the `sequencer-api.log` file. For details, refer to *Logging on page 264*.

3. Contact the Clarity LIMS support team. Provide the relevant information from the troubleshooting steps already performed.

Logging

In addition to updating the Sequencing Log multiline text field on the AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.0) step, the Sequencer API writes a detailed log file to `/opt/gls/clarity/tomcat/current/logs/sequencer-api.log`.

Log messages include the Library Tube ID and Flow Cell ID whenever the messages are related to a sequencing run-related request. In the Clarity LIMS workflows, these IDs are recorded as container names.

Some log messages are not directly related to a sequencing run request, such as downloading the sample sheet. In this case, the file LIMS ID and file name are included in log messages. The sample sheet downloaded by the sequencing system has a name that includes the sequencing container ID (Library Tube for Standard or Flowcell for Xp). The relevant container name search finds most the log messages for this request. However, if all messages for downloading a file are found, then the LIMS ID of the file must be known. The LIMS ID is found in the Clarity LIMS API and, for sample sheets used in a run, in the recipe response (which can also be found in the log file).

Example lines from the log file:

```
2021-06-19 21:32:19.705 INFO --- [] SequencerAPIApplication : Started
SequencerAPIApplication in 7.026 seconds (JVM running for 15.964)
...
2021-06-19 21:34:44.564 INFO --- [Library Tube Id=NV1234567-LIB, Flow Cell
Id=RM123DSXX] LimsApiLookupUtil : Searching for containers with name 'NV1234567-
LIB' or 'RM123DSXX'
....
2021-06-19 21:35:27.158 INFO --- [] FileService : Successfully retrieved file
'NV1234567-LIB.csv' with LIMS ID 40-51 through the API.
```

NovaSeq 6000Dx Integration v1.0 Release Notes

These Release Notes describe the key changes to software components for the Clarity LIMS NovaSeq 6000Dx Integration Package version 1.0.

Compatibility

This version of the integration package is compatible with the following software:

- BaseSpace Clarity LIMS v5.1 and later
- NGS Extensions Package v5.22 and later

- Illumina Preset Protocols (IPP) v2.4.0 and later
- NovaSeq 6000Dx Operating Software (NVOS) v2.3.1

New Features

Features provided with this release of the integration package are as follows.

- An API-based integration between the NovaSeq 6000Dx instrument and BaseSpace Clarity LIMS.
- A direct file and information exchange between Clarity LIMS and NVOS, which includes the following information:
  - Sample sheet generated on Clarity LIMS to NVOS
  - Run setup recipe generated on Clarity LIMS to NVOS
  - Run status updates (RunStarted, RunCompletedSuccessfully, RunEndedByUser, RunErroredOut) from NVOS to Clarity LIMS
  - Run metrics information from NVOS and Clarity LIMS
- Support for both RUO and DX modes.
- The NovaSeqDx v1.0 workflow, delivered directly from IPP v2.4.

Defects Fixed

None

Known Issues

None

Documentation

For detailed information related to this release, refer to the following documents:

- *NovaSeq 6000Dx Integration v1.0 Configuration & User Interaction*
- *NovaSeq 6000Dx Integration v1.0 Validation & Troubleshooting*

NovaSeq 6000 Integrations v3

The Illumina NovaSeq 6000 instrument integration provides preconfigured workflows that map to established lab protocols and steps used with NovaSeq instruments. The integration also supports the associated library prep kits, reagent kits, and assays used in these protocols.

The documents in this section support Illumina NovaSeq 6000 Integration version 3.x.

NovaSeq 6000 Integration v3.6.0 Configuration and User Interaction

The Illumina NovaSeq 6000 Integration Package v3.6.0 supports the integration of Clarity LIMS to NovaSeq 6000 instruments. This documentation describes the integration and includes the following information:

- Preconfigured workflows, protocols, steps, and automations
- Installed components
- Configuration requirements, rules, and constraints

For instructions on validating and troubleshooting the NovaSeq 6000 Integration, refer to *NovaSeq 6000 Integration v3.6.0 Validation and Troubleshooting* on page 331.

> The configuration provided in this integration has been established to support NovaSeq 6000 lab processes. Any configuration changes to protocols or workflows - including renaming protocols, steps, and fields - could break process.

Overview

The integration includes the following features:

- Preconfigured NovaSeq 6000 v3.8 workflow that maps to lab protocols and instrument runs.
- The following preconfigured protocols:
  - Run Format (NovaSeq 6000 v3.8)
  - NovaSeq Standard (NovaSeq 6000 v3.8) (supports the loading of pooled libraries into a library tube)
  - NovaSeq Xp (NovaSeq 6000 v3.8) (supports individual lane loading on the NovaSeq)
  - AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8)
- Automated generation of v1 sample sheet for use with bcl2fastq2 v2.20 analysis software or v2 sample sheet. This file is automatically uploaded to the instrument through the API.

- Automatic validation of run setup information. This information is used to create the run recipe and initiate the run.
- Automated tracking of the NovaSeq sequencing run and parsing of run statistics into Clarity LIMS, including the following features:
  - Progress and metrics of sequencing run
  - Per-instrument sequencing runs (tracked as part of the Run ID field value)
  - Sequencing run parameters
  - Sequencing run data directory location
  - Real-Time Analysis v3 (RTA3) version and other run-specific information
- [Optional] Preconfigured Library Prep Validation v2.3.1 workflow used for validation purposes only. The workflow contains a single-step protocol that models the library prep required to produce normalized libraries that are ready for the NovaSeq 6000 v3.8 workflow. For more information, refer to *NovaSeq 6000 Integration v3.6.0 Validation and Troubleshooting* on page 331. The Library Prep Validation v2.3.1 workflow is available in IPP v2.6 package.

Prerequisites and Assumptions

It is assumed that samples enter the NovaSeq 6000 v3.8 workflow as normalized libraries. It is assumed that the following steps have completed before samples are assigned to the workflow:

- Samples have been accessioned into the Clarity LIMS.
- Samples have been run through QC and library prep.
- Samples have been normalized, and the value is captured in a field called Normalized Molarity (nM).

For more information on sample accessioning, refer to *Sample Accessioning* and *Upload and Modify Samples* in the Getting Started section of the Clarity LIMS Core Functionality documentation.

You can assign samples to workflows automatically, using a routing script, or manually—from the Projects & Samples dashboard. Refer to *Assign and Process Samples* in the Clarity LIMS Core Functionality documentation.

Workflows, Protocols, and Steps

The Illumina NovaSeq 6000 Integration Package v3.6.0 includes the following workflows:

- NovaSeq 6000 v3.8
- Library Prep Validation v2.3.1 (optional, but recommended for validation purposes)

The following table describes the protocols and steps included in these workflows.

Table 100 NovaSeq 6000 Integration Package v3.6.0 Protocols and Steps

Protocol Name	Protocol Purpose	Steps Included in Protocol
	Library Prep Validation v2.3.1 Workflow	
Library Prep Validation v2.3.1	Included for validation purposes only, this protocol models the library prep steps required to advance samples to the Run Format (NovaSeq 6000 v3.8) protocol. The protocol contains a single step - Library Prep Validation v2.3.1. After this step, a routing script sends the samples to the first step of the NovaSeq 6000 v3.8 workflow - Define Run Format (NovaSeq 6000 v3.8).	1. Library Prep Validation v2.3.1
	NovaSeq 6000 v3.8 Workflow	
Run Format (NovaSeq 6000 v3.8)	Allows for the assignment of per sample values for: • Loading Workflow Type: Select either NovaSeq Standard or NovaSeq Xp. • Normalized Molarity: Enter a value for each sample. • Flowcell Type: Select from options SP, S1, S2, or S4. • Final Loading Concentration (pM): Select from options 225 (PCR-free workflows) or 400 (Nano workflows), or enter a different value. Compares the Normalized Molarity value of each sample with the Minimum Molarity value. Routing script sends samples to the NovaSeq Standard or NovaSeq Xp protocol, according to the selected Loading Workflow Type. Samples with Normalized Molarity less than Minimum Molarity are removed from the workflow.	1. Define Run Format (NovaSeq 6000 v3.8)

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Protocol Name	Protocol Purpose	Steps Included in Protocol
NovaSeq Standard (NovaSeq 6000 v3.8)	Samples are pooled and added to the library tube in preparation for the NovaSeq run. The run setup information is validated and a sample sheet is generated. Routing script sends the library tube to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8) protocol.	1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.8) 2. Dilute and Denature (NovaSeq 6000 v3.8)
NovaSeq Xp (NovaSeq 6000 v3.8)	Samples are pooled and added to lanes on the NovaSeq flow cell. The option selected in the Define Run Format (NovaSeq 6000 v3.8) step determines the flow cell type. The run setup information is validated and a sample sheet is generated. Routing script sends flow cell to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8) protocol.	1. Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.8) 2. Dilute, Denature & ExAmp (NovaSeq 6000 v3.8) 3. Load to Flowcell (NovaSeq 6000 v3.8)
AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8)	All samples complete the workflow by going through this protocol. This protocol contains one fully automated step. Do not add samples to the Ice Bucket or start the step. The integration starts the step automatically.	1. AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8)

Validation Workflow

The Library Prep Validation v2.3.1 workflow allows for validation of the system after installation is complete. For details, refer to *NovaSeq 6000 Integration v3.6.0 Validation and Troubleshooting* on page 331.

Step Automations

The following table lists the step automations included in the NovaSeq 6000 v3.8 workflow. Automation details are provided in the documentation for each step.

For details on the validation workflow, refer to *NovaSeq 6000 Integration v3.6.0 Validation and Troubleshooting* on page 331.

Importing either the Library Prep Validation v2.3.1 workflow or the NovaSeq 6000 v3.8 workflows provides a global field named Illumina Universal Sample Identifier. This is a text field that is reserved for CLPA support and is optional. A value is not required for this integration.

Table 101 NovaSeq 6000 v3.8 Workflow Step Automations

Protocol	Step	Automation
1. Run Format (NovaSeq 6000 v3.8)	1. Define Run Format (NovaSeq 6000 v3.8)	Registered Step Started[1] Routing Script & Register Step Completed[2] Set Next Steps
2. NovaSeq Standard (NovaSeq 6000 v3.8)	1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.8)	Calculate Volumes Register Pools & Register Step Completed[1] Register Step Started[1] Set Next Step Validate Inputs Flowcell Type and Single Pool Validate Unique Indexes (Not used. The core Clarity LIMS configuration for pooling handles this functionality.)
	2. Dilute and Denature (NovaSeq 6000 v3.8)	Routing Script & Register Step Completed[2] Validate Library Tube Barcode Validate Run Setup and Generate Sample Sheet Validate Single Input & Register Step Started[2]

Protocol	Step	Automation
3. NovaSeq Xp (NovaSeq 6000 v3.8)	1. Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.8)	Calculate Volumes Register Pools & Register Step Completed[1] Register Step Started[1] Set Next Step Validate Inputs Flowcell Type and Single Pool Validate Unique Indexes (Not used. The core Clarity LIMS configuration for pooling handles this functionality.)
	2. Dilute, Denature & ExAmp (NovaSeq 6000 v3.8)	Calculate Volumes Register Step Completed[1] Set Next Step Validate Inputs Flowcell Type & Register Step Started[2]
	3. Load to Flowcell (NovaSeq 6000 v3.8)	Register Step Completed[1] Validate Flowcell Barcode Validate Inputs and Selected Container & Register Step Started[2] Validate Run Setup and Generate Sample Sheet
4. AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8)	1. AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8)	Register NovaSeq RunCompleted & Register Step Completed[1] Register Step Started & Register NovaSeq Run & Register NovaSeq Run Association & Register NovaSeq RunStarted[1] Update Lane Number Set Next Steps (Not used. By default, the Sequencer API determines this functionality. If necessary, enable the automation and use it to override the next step behavior used by the Sequencer API.)

[1] These automations are for CLPA support only.

[2] These automations are required for the NovaSeq 6000 v3.8 workflow to function properly. These automations contain additional logic needed for CLPA support. If you would like to remove CLPA support, then contact Illumina Support.
Automations not identified with [1] or [2] are required for the NovaSeq 6000 v3.8 to work function properly.

Protocol 1: Run Format (NovaSeq 6000 v3.8)

This protocol sets the Loading Workflow Type and allows the choice of the appropriate Flowcell Type and Final Loading Concentration (pM). After the protocol, a routing script sends the normalized libraries to either the NovaSeq Standard (NovaSeq 6000 v3.8) or the NovaSeq Xp (NovaSeq 6000 v3.8) protocol.

This protocol contains one step: Define Run Format (NovaSeq 6000 v3.8).

Step 1. Define Run Format (NovaSeq 6000 v3.8)

Step input: NTP (normalized libraries)

Step output: None

Register Step Started Automation

Automatically triggered on entry to the step, this automation registers the start time of the step by publishing messages to Clarity LIMS Product Analytics (CLPA) through the ICA LINK service:

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/common/step_started.groovy'
```

This automation is used only for CLPA support.

Set Next Steps Automation

Automatically triggered on exit of the Record Details screen, this automation completes the following actions:

- Sets the next step for samples to REMOVE:

```
nextStep = ::REMOVE::
```

- Calculates the Minimum Molarity using the following formula:

```
input.::Minimum Molarity (nM):: = (5 * input.::Final Loading Concentration (pM)::)/1000
```

- Checks Normalized Molarity value. For samples with no Normalized Molarity value (eg, empty value, not including 0), it generates an error message informing that the field cannot be empty:

```
if (!input.hasValue(::Normalized Molarity (nM)::)) {
  fail(::The Normalized Molarity cannot be empty.::) ;
```


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```
}
```

- Compares each sample Normalized Molarity value with the Minimum Molarity value. If the Normalized Molarity value is lower than the Minimum Molarity value, it sets the samples Loading Workflow Type to [Remove from workflow]. The automation also records a message in the Warning field for the sample:

```
else if (input.::Normalized Molarity (nM):: < input.::Minimum Molarity (nM)::) {
  input.::Warning:: = ::The Normalized Molarity is too low.:: ;
  input.::Loading Workflow Type:: = ::[Remove from workflow]:: ; }
else { input.::Warning:: = ::not applicable:: }
```

At this point there are two options:

- Correct the Normalized Molarity value on the Record Details screen. Edit the Loading Workflow Type field and set it to NovaSeq Standard or NovaSeq Xp, as applicable.
- Complete the protocol without correcting the Normalized Molarity value. In this case, those samples are removed from the workflow.

Routing Script & Register Step Completed Automation

Automatically triggered on exit of the step, this automation invokes the changeWorkflow script, which routes step inputs appropriately.

- Samples with Loading Workflow Type field value = NovaSeq Standard are routed to the NovaSeq 6000 v3.8 workflow. Then, the samples are queued for the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.8) step.
- Samples with Loading Workflow Type field value = NovaSeq Xp are routed to the NovaSeq 6000 v3.8 workflow. Then, the samples are queued for the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.8) step.
- This automation also registers the completion time of the step by publishing messages to CLPA through the ICA LINK service. This is only used for CLPA support.
  Default automation command line is as follows:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -u {username} -p {password} -i {stepURI:v2} -l
{compoundOutputFileLuid0} script:changeWorkflow \
\
--FIELD_NAME 'Loading Workflow Type' \
--FIELD_VALUE 'NovaSeq Standard' \
--WORKFLOW 'NovaSeq 6000 v3.8' \
--STEP 'Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.8)' \
--INPUTS_OR_OUTPUTS 'INPUTS' \
\
--FIELD_NAME 'Loading Workflow Type' \
```


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```
--FIELD_VALUE 'NovaSeq Xp' \
--WORKFLOW 'NovaSeq 6000 v3.8' \
--STEP 'Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.8)' \
--INPUTS_OR_OUTPUTS 'INPUTS' \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/common/step_completed.groovy'"
```

Master Step Fields

The following table lists field configuration details defined on the Define Run Format (NovaSeq 6000 v3.8) master step.

Table 102 Define Run Format (NovaSeq 6000 v3.8) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Comment	Multiline Text		None
Flowcell Type	Text Dropdown	Required Field Custom Entries	Presets • SP • S1 • S2 • S4
Instruction	Text	Read Only	Default • Add Flowcell Type and Loading Workflow Type below
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp

Global Fields

The following table lists the global custom fields that are configured to display on the Define Run Format (NovaSeq 6000 v3.8) step.

Table 103 Define Run Format (NovaSeq 6000 v3.8) Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
Adjusted Per Sample Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Final Loading Concentration (pM)	Numeric Dropdown	Required Field Custom Entries	Decimal places displayed = 0 Presets • 225 • 400
Flowcell Type	Text Dropdown	Required Field	Presets • SP • S1 • S2 • S4
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
Minimum Molarity (nM)	Numeric		Decimal places displayed = 2
Normalized Molarity (nM)	Numeric		Decimal places displayed = 2
Per Sample Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Warning	Text Dropdown	Read Only Custom Entries	The Normalized Molarity (nM) is too low. n/a

User Interaction

Refer to the Step 1: Define Run Format (NovaSeq 6000 v3.8) section in *NovaSeq 6000 Integration v3.6.0 Validation and Troubleshooting* on page 331.

Protocol 2: NovaSeq Standard (NovaSeq 6000 v3.8)

Samples are routed to this protocol when their Loading Workflow Type value is set to NovaSeq Standard. Samples are pooled and added to a library tube in preparation for the NovaSeq run.

At the end of this protocol, a routing script sends the library tube to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8) protocol.

This protocol contains two steps:

- Step 1: Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.8)
- Step 2: Dilute and Denature (NovaSeq 6000 v3.8)

Step 1: Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.8)

In this step, libraries are placed manually into a single pool. Resuspension buffer and reagents are added.

Create only one pool per step.

Step input: NTP (normalized libraries)

Step output: Bulk pool

Validate Unique Indexes automation is not used. The core Clarity LIMS configuration for pooling handles this functionality.

Register Step Started Automation

Automatically triggered on entry to the step, this automation registers the start time of the step by publishing messages to CLPA through the ICA LINK service:

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_started.groovy'
```

This automation is used only for CLPA support.

Validate Inputs Flowcell Type and Single Pool Automation

Automatically triggered on exit of the Pooling screen, this automation completes the following actions:

- Checks that all samples in the pool have the same Flowcell Type assigned to them.
- Checks that only one pool has been created.
- Changes the value of error and logging messages to reference the type of pool passed as bulk pool.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar \
script:validate_flowcell_for_input_pools \
  -i {stepURI:v2} \
  -u {username} \
  -p {password} \
```

```
  -l {compoundOutputFileLuid1} \
  -validateSingleOutput true \
  -poolType bulk"
```

Calculate Volumes Automation

Automatically triggered when selecting the Calculate Volumes button on the Record Details screen, this automation completes the following actions:

- Calculates the number of samples in the pool.

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: + 1
```

- Sets the value of the Bulk Pool Volume (µl), and PhiX Volume (µl) field based on the selected Flowcell Type.

```
step.::PhiX Volume (ul):: = !step.hasValue(::% PhiX (2.5nM) Spike-In::) ? 0 :
step.::% PhiX (2.5nM) Spike-In::;
if (input.::Flowcell Type:: == ::SP::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 100;
step.::PhiX Volume (ul):: *= 0.6
};
if (input.::Flowcell Type:: == ::S1::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 100;
step.::PhiX Volume (ul):: *= 0.6
};
if (input.::Flowcell Type:: == ::S2::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 150;
step.::PhiX Volume (ul):: *= 0.9
};
if (input.::Flowcell Type:: == ::S4::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 310;
step.::PhiX Volume (ul):: *= 1.9
};
if (step.::PhiX Volume (ul):: == 0) {
step.::PhiX Volume (ul):: = ::::
};
```

- Calculates the Per Sample Volume (µl) to be added to the pool.

```
input.::Per Sample Volume (ul):: = ( ( (input.::Final Loading Concentration
(pM):: * 5 / 1000) / input.::Normalized Molarity (nM):: ) * step.::Bulk Pool
Volume (ul):: ) / step.::Number of Samples in Pool::
```

For accurate pipetting of each sample in a pool, the Per Sample Volume (µl) value must be greater than or equal to the Minimum Per Sample Volume (µl). The default value (set at 5) can be edited.

Assuming the default Minimum Per Sample Volume (µl) value of 5, completes the following steps for a given batch:

1. If the smallest Per Sample Volume (µl) value is less than 5, automatically assigns a value of 5 to the Adjusted Per Sample Volume (µl) field.
2. Adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch. This adjustment is based on the ratio used to increase the lowest value to 5.

- Calculates the Total Sample Volume (µl) field value.

```
step.::Total Sample Volume (µl):: = step.::Total Sample Volume (µl):: +
input.::Adjusted Per Sample Volume (µl)::
```

- If the Total Sample Volume is less than the Bulk Pool Volume, the automation calculates the RSB Volume (µl) field value.

```
if (step.::Total Sample Volume (ul):: >= step.::Bulk Pool Volume (ul)::)
{output.::RSB Volume (ul):: = 0}
else {output.::RSB Volume (ul):: = step.::Bulk Pool Volume (ul):: - step.::Total
Sample Volume (ul)::} ;
```

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs.

```
output.::Flowcell Type:: = input.::Flowcell Type:: ;
output.::Loading Workflow Type:: = input.::Loading Workflow Type:: ;
```

- Sets the Volume of Pool to Denature (µl) value and calculates the NaOH Volume (µl) and Tris-HCl Volume (µl) values based on the Flowcell Type:

```
if ( input.::Flowcell Type:: == ::SP:: ) { output.::Volume of Pool to Denature
(ul):: = 100 ; output.::NaOH Volume (ul):: = 25 ; output.::Tris-HCl Volume
(ul):: = 25 } ;
if ( input.::Flowcell Type:: == ::S1:: ) { output.::Volume of Pool to Denature
(ul):: = 100 ; output.::NaOH Volume (ul):: = 25 ; output.::Tris-HCl Volume
(ul):: = 25 } ;
if ( input.::Flowcell Type:: == ::S2:: ) { output.::Volume of Pool to Denature
(ul):: = 150 ; output.::NaOH Volume (ul):: = 37 ; output.::Tris-HCl Volume
(ul):: = 38 } ;
if ( input.::Flowcell Type:: == ::S4:: ) { output.::Volume of Pool to Denature
(ul):: = 310 ; output.::NaOH Volume (ul):: = 77 ; output.::Tris-HCl Volume
(ul):: = 78 }
```


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- Uses the `NovaSeq_Standard_Bulk_Pool1.csv`, `NovaSeq_Standard_Bulk_Pool2.csv`, and `NovaSeq_Standard_Bulk_Pool3.csv` template files to generate a single CSV file containing information about the pool and the samples it contains. The generated file is available for download on the Step Setup screen of Dilute and Denature (NovaSeq 6000 v3.8).

```
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Standard_Bulk_
Pool1.csv \
-o 1.csv \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Standard_Bulk_
Pool2.csv \
-o 2.csv \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Standard_Bulk_
Pool3.csv \
-o 3.csv \
&& cat 1.csv 2.csv 3.csv > {compoundOutputFileLuid0}.csv
```

- Resets the Total Sample Volume (µl) and Number of Samples in Pool field values so that the automation is idempotent.

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: - 1;
step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: -
input.::Adjusted Per Sample Volume (ul)::
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, the following automation sets the next step for samples to ADVANCE. The samples advance to the Dilute and Denature (NovaSeq 6000 v3.8) step:

```
nextStep = ::ADVANCE::
```

Register Pools & Register Step Completed Automation

Automatically triggered on exit of the step, the following automation completes the following actions:

- Registers the pool and library information used by the Clarity LIMS workflow. Samples in the pool are assumed to have gone through library preparation.
- Registers the completion time of the step.

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These actions are done by publishing messages to CLPA through the ICA LINK service. This automation is used only for CLPA support.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/pooling.groovy' && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_completed.groovy'
```

Master Step Fields

The following table lists configuration details defined on the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.8) step.

Table 104 Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.8) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
% PhiX (2.5 nM) Spike-In	Numeric		Range = 0–100
Bulk Pool Volume (µl) (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 2
Number of Flowcells to Sequence	Numeric	Required Field	Range = 1–10 Decimal places displayed = 0
Minimum Per Sample Volume (µl)	Numeric	Required Field	Decimal places displayed = 2 Default • 5
Number of Samples in Pool (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 0 Default • 0

Field Name	Field Type	Options	Additional Options and Dropdown Items
PhiX Volume (µl) (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 2
Total Sample Volume (µl) (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 2 Default • 0

Global Fields

The following table lists the global custom fields that are configured to display on the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.8) step.

Table 105 Global Custom Fields Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
Flowcell Type	Text Dropdown	Required Field	Presets • SP • S1 • S2 • S4
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
NaOH Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
RSB Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Tris-HCl Volume (µl)	Numeric	Read Only	Decimal places displayed = 2

Field Name	Field Type	Options	Additional Options and Dropdown Items
Volume of Pool to Denature (µl) Used in Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.8) step only. Displays on Record Details screen and in the generated CSV file.	Numeric	Read Only	Decimal places displayed = 0

User Interaction

Refer to the Step 1: Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.8) section in *NovaSeq 6000 Integration v3.6.0 Validation and Troubleshooting* on page 331.

Step 2: Dilute and Denature (NovaSeq 6000 v3.8)

In this step, the addition of NaOH, Tris-HCl, and Resuspension Buffer (RSB) denatures and dilutes the pooled samples. Manually place the pooled samples into the library tube for the NovaSeq run.

In addition, this step validates the run setup information and generates the sample sheet file.

Step input: Bulk pool

Step output: Library tube

Validate Single Input & Register Step Started Automation

Automatically triggered at the beginning of the step, this automation completes the following actions:

- Checks that there is only one container input to the step.

```
script:validateSampleCount -min 1 -max 1
```

- Registers the start time of the step by publishing messages to CLPA through the ICA LINK service. This script is used only for CLPA support.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_
started.groovy'
```

Validate Library Tube Barcode Automation

Automatically triggered on exit of the Placement screen, this automation completes the following actions:

- Validates the library tube barcode to ensure it conforms to the barcode mask [A-Z]{2}[0-9]{7}-[A-Z]{3}:

```
if (!output.container.name.matches(::[A-Z]{2}[0-9]{7}-[A-Z]{3}::)) {
fail(::Invalid Library Tube Barcode. Verify and try again.::)
}
```

- Copies Flowcell Type and Loading Workflow Type field values from step inputs to outputs.

```
output.::Flowcell Type:: = input.::Flowcell Type:: ;
output.::Loading Workflow Type:: = input.::Loading Workflow Type::
```

Validate Run Setup and Generate Sample Sheet Automation

The script definition portion of Validate Run Setup has been moved into the Validation Script custom field due to the 4000 character limit.

- If modification is required on Validate Run Setup script, perform the changes directly on the Validation Script custom field default value.
- The script definition portion of the Generate Sample Sheet remains untouched.

Triggered by a button on the Record Details screen, this automation completes the following actions:

- Copies the Flowcell Type from the step input to the Run Mode field (hidden):

```
step.::Run Mode:: = input.::Flowcell Type::
```

- Validates the parameters entered on the Record Details screen, which are used to set up the run and generate the sample sheet file. For more information, refer to the *Respond to Validation / Recipe Request Call from Instrument* section of *NovaSeq 6000 Run* on page 317.
  - Experiment Name can only contain alphanumeric, dash, or underscore characters. Spaces are not permitted.
  - When Workflow Type = No Index, both Index Read 1 and Index Read 2 must be zero.
  - When Workflow Type = Single Index, Index Read 1 must be greater than zero, and Index Read 2 must be zero.
  - When Workflow Type = Dual Index, both Index Read 1 and Index Read 2 must be greater than zero.

```
if(!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) {
fail(::Experiment Name contains prohibited characters. Allowed characters are:
a-z, A-Z, 0-9, -, and _::)
};
if (step.::Workflow Type:: == ::No Index::) {
```

```
if (step.::Index Read 1:: != 0 || step.::Index Read 2:: != 0) {
fail(::Index Read 1 and Index Read 2 must be 0 if No Index is selected.::)
}
} else if (step.::Workflow Type:: == ::Single Index::) {
if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) {
fail(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if Single
Index is selected.::)
}
} else {
if (step.::Index Read 1:: == 0 || step.::Index Read 2:: == 0) {
fail(::Index Read 1 and Index Read 2 must be greater than 0 if Dual Index is
selected.::)
}
};
```

- Checks the Paired End and Read 2 Cycles field values.
  - If Paired End = True and Read 2 Cycles value is zero, generates an error.
  - If Paired End = False, Read 2 Cycles must be zero and UMI - Read 2 Length and UMI - Read 2 Start From Cycle must be empty.

```
if (step.::Paired End::.toBoolean()) {
if (step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) {
fail(::Read 1 Cycles and Read 2 Cycles must be greater than 0 if Paired End is
True.::)
}
} else {
if (step.::Read 2 Cycles:: != 0) {
fail(::Read 2 Cycles must be 0 if Paired End is False.::)
};
if (step.hasValue(::UMI - Read 2 Length::) || step.hasValue(::UMI - Read 2 Start
From Cycle::)) {
fail(::UMI - Read 2 Length and UMI - Read 2 Start From Cycle cannot be defined
if Paired End is False.::)
}
};
```

- Checks the Flowcell field value.
  - If Flowcell Type value is not SP, checks that the values of Read 1 Cycles and Read 2 Cycles are each 151 or less. If the value is greater than 151, an error message is generated.

```
if (input.::Flowc ell Type::!=::SP:: && step.::Read 1 Cycles:: > 151) {
fail(::Read 1 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
};
```


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```
if (input.::Flowcell Type::!=::SP:: && step.::Read 2 Cycles:: > 151) {
fail(::Read 2 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
};
```

- Validates UMI Settings.
  - If UMI - Read 1 Length has value, then UMI - Read 1 Start From Cycle must have value.
  - If UMI - Read 1 Length has no value, then UMI - Read 1 Start From Cycle must not have value.
  - If UMI - Read 2 Length has value, then UMI - Read 2 Start From Cycle must have value.
  - If UMI - Read 2 Length has no value, then UMI - Read 2 Start From Cycle must not have value.
  - If UMI - Read 1 Length has no value, then UMI - Read 2 Length must not have value.
  - If UMI - Read 1 Start From Cycle has no value, then UMI - Read 2 Start From Cycle must not have value.

```
if (step.hasValue(::UMI - Read 1 Length::) && !step.hasValue(::UMI - Read 1
Start From Cycle::)) {
fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 1
Length is greater than 0.::)
};
if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 1
Start From Cycle::)) {
fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 1 Start From
Cycle is greater than 0.::)
};
if (step.hasValue(::UMI - Read 2 Length::) && !step.hasValue(::UMI - Read 2
Start From Cycle::)) {
fail(::UMI - Read 2 Start From Cycle must be greater than 0 if UMI - Read 2
Length is greater than 0.::)
};
if (!step.hasValue(::UMI - Read 2 Length::) && step.hasValue(::UMI - Read 2
Start From Cycle::)) {
fail(::UMI - Read 2 Length must be greater than 0 if UMI - Read 2 Start From
Cycle is greater than 0.::)
};
if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 2
Length::)) {
fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 2 Length is
greater than 0.::)
};
if (!step.hasValue(::UMI - Read 1 Start From Cycle::) && step.hasValue(::UMI -
Read 2 Start From Cycle::)) {
```


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```
fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 2
Start From Cycle is greater than 0.::)
};
```

- Validate Analysis Software Version to ensure it is a valid version string.

```
if (step.hasValue(::Analysis Software Version::) && !step.::Analysis Software
Version::.matches(::^(\\d+)(\\.\\d+)*$::)) {
fail(::Analysis Software Version contains prohibited characters. Allowed
characters are 0-9 and period. It shall start and end with numbers and separated
by single period e.g. 3.8.4::)
};
```

- Validate Override Cycles value to contain only Y, N, I, U, 0-9, and semicolon characters.

```
if (step.hasValue(::Override Cycles::) && !step.::Override Cycles::.matches(::
[YNIU0-9;]+::)) {
fail(::Override Cycles contains prohibited characters. Allowed characters are:
Y, N, I, U, 0-9 and ;. Example: N1Y150;I8;I7N1;Y141U10.::)
};
```

- Outputs the Settings Header in sample sheet when UMI - Read 1 Length or UMI - Read 2 Length have value.

```
step.::Settings Header:: = step.hasValue(::UMI - Read 1 Length::) ||
step.hasValue(::UMI - Read 2 Length::) ? ::[Settings]:: : ::::;
```

- Sets the next step for samples to REMOVE.

```
nextStep = ::REMOVE::
```

- Generates the sample sheet and attaches it to the step. For more information, refer to *Sample Sheet Generation*. Sample sheet template used depends on the selected value of Samplesheet Format and/or Reverse Complement Workflow.

```
&& if [ '{udf:Samplesheet Format}' == 'V2' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Samplesheet_v2.csv'; \
elif [ '{udf:Reverse Complement Workflow}' == 'true' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Reverse_Complement_Samplesheet.csv'; \
elif [ '{udf:Reverse Complement Workflow}' == 'false' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Samplesheet.csv'; \
else echo 'UDF Reverse Complement Workflow is empty or undefined.'; exit -1; fi
\
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/$templateFilename \
-o {compoundOutputFileLuid0}.csv \
```

```
-q true \
-destLIMSID {compoundOutputFileLuid0} \
-l {compoundOutputFileLuid1}"
```

Routing Script & Register Step Completed Automation

Automatically triggered on exit of the step, this automation completes the following actions:

- Invokes the changeWorkflow script. This script routes step outputs to the NovaSeq 6000 v3.8 workflow and queues them for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8) step.

```
script:changeWorkflow \
--FIELD_NAME 'N/A' \
--FIELD_VALUE 'N/A' \
--WORKFLOW 'NovaSeq 6000 v3.8' \
--STEP 'AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8)' \
--INPUTS_OR_OUTPUTS 'OUTPUTS' \
```

- Registers the completion time of the step by publishing messages to CLPA through the ICA LINK service. This script is used only for CLPA support.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_
completed.groovy'
```

Master Step Fields

The following table lists field configuration details defined on the Dilute and Denature (NovaSeq 6000 v3.8) step. These fields are required for sample sheet and JSON file generation.

Table 106 Dilute and Denature (NovaSeq 6000 v3.8) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Analysis Software Version	Text		
BaseSpace Sequence Hub Configuration	Text Dropdown	Required Field	Presets • Not Used • Run Monitoring Only • Run Monitoring and Storage

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Field Name	Field Type	Options	Additional Options and Dropdown Items
Custom Recipe Path	Text		Not applicable
Experiment Name	Text	Required Field	Not applicable
Index Read 1	Numeric Dropdown	Required Field Custom Entries	Range = 0–20 Decimal places displayed = 0 Presets • 0 • 6 • 8
Index Read 2	Numeric Dropdown	Required Field Custom Entries	Range = 0–20 Decimal places displayed = 0 Presets • 0 • 6 • 8
Output Folder	Text	Required Field	Not applicable
Override Cycles	Text		Not applicable
Paired End	Text Dropdown	Required Field	Presets • True • False
Read 1 Cycle	Numeric Dropdown	Required Field Custom Entries	Range = 1–251 Decimal places displayed = 0 Presets • 251* • 151 • 101 • 51 *Value of 251 is only supported for SP flow cell type. For all other cell types, maximum value is 151.

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Field Name	Field Type	Options	Additional Options and Dropdown Items
Read 2 Cycle	Numeric Dropdown	Required Field Custom Entries	Range = 0–251 Decimal places displayed = 0 Presets • 251* • 151 • 101 • 51 *Value of 251 is only supported for SP flow cell type. For all other cell types, maximum value is 151.
Reverse Complement Workflow	Toggle Switch	Required Field	Default • Yes
Run Mode (Not displayed in user interface)	Text Dropdown	Read Only	Presets • SP • S1 • S2 • S4
Samplesheet Format	Text Dropdown	Required Field	Presets • V1 (default) • V2
Settings Header (not displayed in user interface)	Text	Read Only	Not applicable
UMI—Read 1 Length	Numeric		Range = 1
UMI—Read 1 Start From Cycle	Numeric		Range = 1
UMI—Read 2 Length	Numeric		Range = 1
UMI—Read 2 Start From Cycle	Numeric		Range = 1
Use Custom Index Read 1 Primer	Toggle Switch		Default • None Set

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Field Name	Field Type	Options	Additional Options and Dropdown Items
Use Custom Read 1 Primer	Toggle Switch		Default • None Set
Use Custom Read 2 Primer	Toggle Switch		Default • None Set
Use Custom Recipe	Toggle Switch	Required Field	Default • No
Validation Script	Multiline Text	Required Field Read Only	Default value is provided in the drop-down section that follows the table. Do not remove this field as it is used by Validate Run Setup and Generate Sample Sheet automation script.
Workflow	Text	Read Only	Default • GenerateFASTQ
Workflow Type	Text Dropdown	Required Field	Presets • No Index • Single Index • Dual Index • Custom

Validation Script

```
step.::Run Mode:: = input.::Flowcell Type::; if (!step.::Experiment
Name::.matches(::[a-zA-Z0-9-_]+::)) { fail(::Experiment Name contains prohibited
characters. Allowed characters are: a-z, A-Z, 0-9, -, and _::) }; if
(step.::Workflow Type:: == ::No Index::) { if (step.::Index Read 1:: != 0 ||
step.::Index Read 2:: != 0) { fail(::Index Read 1 and Index Read 2 must be 0 if
No Index is selected.::) } } else if (step.::Workflow Type:: == ::Single
Index::) { if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) { fail
(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if Single
Index is selected.::) } } else { if (step.::Index Read 1:: == 0 || step.::Index
```

```
Read 2:: == 0) { fail(::Index Read 1 and Index Read 2 must be greater than 0 if
Dual Index is selected.::) } }; if (step.::Paired End::.toBoolean()) { if
(step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) { fail(::Read 1
Cycles and Read 2 Cycles must be greater than 0 if Paired End is True.::) } }
else { if (step.::Read 2 Cycles:: != 0) { fail(::Read 2 Cycles must be 0 if
Paired End is False.::) }; if (step.hasValue(::UMI - Read 2 Length::) ||
step.hasValue(::UMI - Read 2 Start From Cycle::)) { fail(::UMI - Read 2 Length
and UMI - Read 2 Start From Cycle cannot be defined if Paired End is False.::) }
}; if (input.::Flowcell Type:: != ::SP:: && step.::Read 1 Cycles:: > 151) { fail
(::Read 1 Cycles must not be larger than 151 if it is not SPrime Flowcell::) };
if (input.::Flowcell Type:: != ::SP:: && step.::Read 2 Cycles:: > 151) { fail
(::Read 2 Cycles must not be larger than 151 if it is not SPrime Flowcell::) };
if (step.hasValue(::UMI - Read 1 Length::) && !step.hasValue(::UMI - Read 1
Start From Cycle::)) { fail(::UMI - Read 1 Start From Cycle must be greater than
0 if UMI - Read 1 Length is greater than 0.::) }; if (!step.hasValue(::UMI -
Read 1 Length::) && step.hasValue(::UMI - Read 1 Start From Cycle::)) { fail
(::UMI - Read 1 Length must be greater than 0 if UMI - Read 1 Start From Cycle
is greater than 0.::) }; if (step.hasValue(::UMI - Read 2 Length::) &&
!step.hasValue(::UMI - Read 2 Start From Cycle::)) { fail(::UMI - Read 2 Start
From Cycle must be greater than 0 if UMI - Read 2 Length is greater than 0.::)
}; if (!step.hasValue(::UMI - Read 2 Length::) && step.hasValue(::UMI - Read 2
Start From Cycle::)) { fail(::UMI - Read 2 Length must be greater than 0 if UMI
- Read 2 Start From Cycle is greater than 0.::) }; if (!step.hasValue(::UMI -
Read 1 Length::) && step.hasValue(::UMI - Read 2 Length::)) { fail(::UMI - Read
1 Length must be greater than 0 if UMI - Read 2 Length is greater than 0.::) };
if (!step.hasValue(::UMI - Read 1 Start From Cycle::) && step.hasValue(::UMI -
Read 2 Start From Cycle::)) { fail(::UMI - Read 1 Start From Cycle must be
greater than 0 if UMI - Read 2 Start From Cycle is greater than 0.::) }; if
(step.hasValue(::Analysis Software Version::) && !step.::Analysis Software
Version::.matches(::^(\\d+)(\\.\\d+)*$::)) { fail(::Analysis Software Version
contains prohibited characters. Allowed characters are 0-9 and period. It shall
start and end with numbers and separated by single period e.g. 3.8.4::) }; if
(step.hasValue(::Override Cycles::) && !step.::Override Cycles::.matches(::
[YNIU0-9;]+::)) { fail(::Override Cycles contains prohibited characters. Allowed
characters are: Y, N, I, U, 0-9 and ;. Example: N1Y150;I8;I7N1;Y141U10.::) };
step.::Settings Header:: = step.hasValue(::UMI - Read 1 Length::) ||
step.hasValue(::UMI - Read 2 Length::) ? ::[Settings]:: : ::::; nextStep =
::REMOVE::
```


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Global Fields

The following table lists the global custom fields that are configured to display on the Dilute and Denature (NovaSeq 6000 v3.8) step.

Table 107 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
Flowcell Type	Text Dropdown	Required Field	Presets • SP • S1 • S2 • S4
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User Interaction

Refer to the Step 2: Dilute and Denature (NovaSeq 6000 v3.8) section in *NovaSeq 6000 Integration v3.6.0 Validation and Troubleshooting* on page 331.

Protocol 3: NovaSeq Xp (NovaSeq 6000 v3.8)

Samples are routed to this protocol when their Loading Workflow Type value is set to NovaSeq Xp.

Samples are pooled and added to lanes on the NovaSeq flow cell type selected in the Define Run Format (NovaSeq 6000 v3.8) step. At the end of this protocol, the flow cell is sent to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8) protocol.

This protocol contains the following three steps:

- Step 1: Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.8)
- Step 2: Dilute, Denature & ExAmp (NovaSeq 6000 v3.8)
- Step 3: Load to Flowcell (NovaSeq 6000 v3.8)

Step 1: Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.8)

Manually place libraries into a pool.

Create only one pool per step.

Step input: NTP (normalized libraries)

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Step output: Bulk pool

Validate Unique Indexes automation is not used. The core Clarity LIMS configuration for pooling handles this functionality.

Register Step Started Automation

Automatically triggered on entry to the step, this automation registers the start time of the step by publishing messages to CLPA through the ICA LINK service:

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/common/step_started.groovy'
```

This automation is used only for CLPA support.

Validate Inputs Flowcell Type and Single Pool Automation

Automatically triggered on exit of the Pooling screen, this automation completes the following actions:

- Checks that all samples in the pool have the same Flowcell Type assigned to them.
- Checks that only one pool has been created.
- Changes the value of error and logging messages to reference the type of pool passed as 'bulk' pool.

```
/opt/gls/clarity/bin/java \
-jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar \
script:validate_flowcell_for_input_pools \
-i {stepURI:v2} \
-u {username} \
-p {password} \
-l {compoundOutputFileLuid1} \
-validateSingleOutput true \
-poolType bulk"
```

Calculate Volumes Automation

Triggered when the Calculate Volumes button is selected on the Record Details screen, this automation completes the following actions:

- Calculates the number of samples in the pool.

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: + 1
```

- Sets the value of the Bulk Pool Volume (µl) and PhiX Volume (µl) field, based on the selected Flowcell Type.

```
step.::PhiX Volume (ul):: = !step.hasValue(::% PhiX (0.25nM) Spike-In::) ? 0 :
step.::% PhiX (0.25nM) Spike-In::;
if (input.::Flowcell Type:: == ::SP::) {
step.::Bulk Pool Volume (ul):: = step.'Number of Lanes to Sequence' * 18;
step.::PhiX Volume (ul):: *= 0.7;
};
if (input.::Flowcell Type:: == ::S1::) {
step.::Bulk Pool Volume (ul):: = step.'Number of Lanes to Sequence' * 18;
step.::PhiX Volume (ul):: *= 0.7;
};
if (input.::Flowcell Type:: == ::S2::) {
step.::Bulk Pool Volume (ul):: = step.'Number of Lanes to Sequence' * 22;
step.::PhiX Volume (ul):: *= 0.8;
};
if (input.::Flowcell Type:: == ::S4::) {
step.::Bulk Pool Volume (ul):: = step.'Number of Lanes to Sequence' * 30;
step.::PhiX Volume (ul):: *= 1.1;
};
if (step.::PhiX Volume (ul):: == 0) {
step.::PhiX Volume (ul):: = ::::;
};
```

- Calculates the Per Sample Volume (µl) to be added to the pool.

```
input.::Per Sample Volume (µl)::= (((input.::Final Loading Concentration (pM)::*
5/ 1000) / input.::Normalized Molarity (nM)::) * step.::Bulk Pool Volume (µl)::)
/ step.::Number of Samples in Pool::
```

> For accurate pipetting of each sample in a pool, the Per Sample Volume (µl) value must be greater than or equal to the Minimum Per Sample Volume (µl). The default value (set at 5) can be edited.
>
> Assuming the default Minimum Per Sample Volume (µl) value of 5, completes the following steps for a given batch:
>
> 1. If the smallest Per Sample Volume (µl) value is less than 5, automatically assigns a value of 5 to the sample Adjusted Per Sample Volume (µl) field.
> 2. Adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch. This adjustment is based on the ratio used to increase the lowest value to 5.

- Calculates the Total Sample Volume (µl) field value.

```
step.::Total Sample Volume (µl):: = step.::Total Sample Volume (µl):: +
input.::Adjusted Per Sample Volume (µl)::
```

- If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) field value.

```
if (step.::Total Sample Volume (µl):: >= step.::Bulk Pool Volume (µl)::)
{output.::RSB Volume (µl):: = 0} else {output.::RSB Volume (µl):: = step.::Bulk
Pool Volume (µl):: - step.::Total Sample Volume (µl)::} ;
```

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs.

```
output.::Flowcell Type::= input.::Flowcell Type::;output.::Loading Workflow
Type::= input.::Loading Workflow Type::;
```

- Uses the `NovaSeq_Xp_Bulk_Pool.csv` and `NovaSeq_Xp_Bulk_Pool2.csv` template files to generate a single CSV file containing information about the bulk pool and the samples it contains. The generated file is available for download on the Step Setup screen of the Dilute, Denature & ExAmp (NovaSeq 6000 v3.8) step.

```
&& /opt/gls/clarity/bin/java \-jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar \
  -i {stepURI:v2} \
  -u {username} \
  -p {password} \
  -l {compoundOutputFileLuid1} \ script:driver_file_generator \
  -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_Bulk_
Pool.csv \
  -o {compoundOutputFileLuid0}.csv \ script:driver_file_generator \
  -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_Bulk_
Pool2.csv \
  -o append.csv \
&& cat append.csv >> {compoundOutputFileLuid0}.csv"
```

- Resets the Total Sample Volume (µl) and Number of Samples in Pool field values so that the automation is idempotent.

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: - 1;
step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: -
input.::Adjusted Per Sample Volume (ul)::
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation completes the following actions:

- Copies the Flowcell Type values from the step inputs to the step outputs.
- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol.

```
output.::Flowcell Type:: = input.::Flowcell Type:: ; nextStep = ::ADVANCE::
```

Register Pools & Register Step Completed Automation

Automatically triggered on exit of the step, the following automation completes the following actions:

- Registers the pool and library information used by the Clarity LIMS workflow. Samples in the pool are assumed to have gone through library preparation.
- Registers the completion time of the step.

These actions are done by publishing messages to CLPA through the ICA LINK service. This automation is used only for CLPA support.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/pooling.groovy' && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_completed.groovy'
```

Master Step Fields

The following table lists field configuration details defined on the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.8) step.

Table 108 Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.8) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
% PhiX (0.25nM) Spike-in	Numeric		Range = 0–100
Bulk Pool Volume (µl) (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 2
Minimum Per Sample Volume (µl)	Numeric	Required Field	Decimal places displayed = 2 Default • 5
Number of Lanes to Sequence	Numeric	Required Field	Decimal places displayed = 0

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Field Name	Field Type	Options	Additional Options and Dropdown Items
Number of Samples in Pool (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 0 Default • 0
PhiX Volume (µl) (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 2
Total Sample Volume (µl) (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 0 Default • 0

Global Fields

The following table lists the global custom fields that are configured to display on the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.8) step.

Table 109 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
RSB Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Flowcell Type	Text Dropdown	Required Field	Presets • SP • S1 • S2 • S4
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User Interaction

Refer to the Step 1: Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.8) section in *NovaSeq 6000 Integration v3.6.0 Validation and Troubleshooting* on page 331.

Step 2: Dilute, Denature & ExAmp (NovaSeq 6000 v3.8)

In this step, the addition of DPX, NaOH, Tris-HCl, and RSB denatures and dilutes the pooled samples. Manually create working pools based on the number of lanes that you want to sequence.

Step input: Bulk pool

Step output: Working pool - variable number, choose how many working pools to create per bulk pool

Validate Inputs Flowcell Type & Register Step Started Automation

Automatically triggered at the beginning of the step, this automation invokes the `validate_flowcell_for_input_pools` script. This script completes the following actions:

- Checks the inputs to the step and validates that the Flowcell Type field is set to a valid value (SP, S1, S2, or S4). The automation also validates that all inputs have the same value for this field.
- Checks that the container type selected matches the value in the Flowcell Type field.

```
script:validate_flowcell_for_input_pools \
-i {stepURI:v2} \
-u {username} \
-p {password} \
-l {compoundOutputFileLuid1}" \
```

- Registers the start time of the step by publishing messages to CLPA through the ICA LINK service:

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/common/step_started.groovy'
```

  This automation is used only for CLPA support.

Calculate Volumes Automation

Automatically triggered on entry to of the Record Details screen, this automation sets the value of the following fields based on the Flowcell Type:

- BP Aliquot Volume (µl)
- NaOH Volume (µl)
- Tris-HCl Volume (µl)
- DPX1 Volume (µl)

- DPX2 Volume (µl)
- DPX3 Volume (µl)
- Mastermix per Lane (µl)

```
if ( input.::Flowcell Type:: == ::SP:: ) {
output.::BP Aliquot Volume (ul):: = 18 ;
output.::NaOH Volume (ul):: = 4;
output.::Tris-HCl Volume (ul):: = 5;
step.::DPX1 Volume (ul):: = 126 ;
step.::DPX2 Volume (ul):: = 18 ;
step.::DPX3 Volume (ul):: = 66 ;
output.::Mastermix per Lane (ul):: = 63
};
if (input.::Flowcell Type:: == ::S1::) {
output.::BP Aliquot Volume (ul):: = 18;
output.::NaOH Volume (ul):: = 4;
output.::Tris-HCl Volume (ul):: = 5;
step.::DPX1 Volume (ul):: = 126;
step.::DPX2 Volume (ul):: = 18;
step.::DPX3 Volume (ul):: = 66;
output.::Mastermix per Lane (ul):: = 63
};
if (input.::Flowcell Type:: == ::S2::) {
output.::BP Aliquot Volume (ul):: = 22;
output.::NaOH Volume (ul):: = 5;
output.::Tris-HCl Volume (ul):: = 6;
step.::DPX1 Volume (ul):: = 126;
step.::DPX2 Volume (ul):: = 18;
step.::DPX3 Volume (ul):: = 66;
output.::Mastermix per Lane (ul):: = 77
};
if (input.::Flowcell Type:: == ::S4::) {
output.::BP Aliquot Volume (ul):: = 30;
output.::NaOH Volume (ul):: = 7;
output.::Tris-HCl Volume (ul):: = 8;
step.::DPX1 Volume (ul):: = 315;
step.::DPX2 Volume (ul):: = 45;
step.::DPX3 Volume (ul):: = 165;
output.::Mastermix per Lane (ul):: = 105
};
```


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The automation also completes the following actions:

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs.

```
output.::Flowcell Type::= input.::Flowcell Type::;
output.::Loading Workflow Type::= input.::Loading Workflow Type::;
```

- Uses the `NovaSeq_Xp_Working_Pool.csv` and `NovaSeq_Xp_Working_Pool2.csv` template files to generate a single CSV file. This file contains information about the DPX volume and the volume of BP Aliquot, Mastermix, NaOH, and Tris-HCl to add per working pool. The generated file is available for download on the Step Setup screen of the Load to Flowcell (NovaSeq 6000 v3.8) step.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_Working_
Pool.csv \
-o {compoundOutputFileLuid0}.csv \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_Working_
Pool2.csv \
-o append.csv \
&& cat append.csv >> {compoundOutputFileLuid0}.csv"
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation completes the following actions:

- Copies the Flowcell Type values from the step inputs to the step outputs.
- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ; nextStep = ::ADVANCE::
```

Register Step Completed Automation

Automatically triggered on exit of the step, this automation registers the completion time of the step by publishing messages to CLPA through the ICA LINK service. This script is used only for CLPA support.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_
completed.groovy'
```

Master Step Fields

The following table lists field configuration details defined on the Load to Flowcell (NovaSeq 6000 v3.8) step. A script sets these field values. The values are not editable while running the step.

Table 110 Dilute, Denature & ExAmp (NovaSeq v3.8) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
DPX1 Volume (µl)	Numeric	Read Only	Decimal places displayed = 0
DPX2 Volume (µl)	Numeric	Read Only	Decimal places displayed = 0
DPX3 Volume (µl)	Numeric	Read Only	Decimal places displayed = 0

Global Fields

The following table lists the global custom fields that are configured to display on the Dilute, Denature & ExAmp (NovaSeq 6000 v3.8) step.

Table 111 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
BP Aliquot Volume (µl)	Numeric	Read Only	Decimal places displayed = 0
Flowcell Type	Text Dropdown	Required Field	Presets • SP • S1 • S2 • S4
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

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Field Name	Field Type	Options	Additional Options and Dropdown Items
Mastermix per Lane (µl)	Numeric	Read Only	Decimal places displayed = 0
NaOH Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Tris-HCl Volume (µl)	Numeric	Read Only	Decimal places displayed = 2

User Interaction

Refer to the Step 2: Dilute, Denature & ExAmp (NovaSeq 6000 v3.8) section in *NovaSeq 6000 Integration v3.6.0 Validation and Troubleshooting* on page 331.

Step 3: Load to Flowcell (NovaSeq 6000 v3.8)

In this step, scan the flow cell barcode into the Clarity LIMS. Then, manually place the working pools into the lanes of the flow cell for the NovaSeq run. This step validates the run setup information and generates the sample sheet file.

Step input: Working pool

Step output: Flow cell (output containers: SP, S1, and S2 with 2 lanes, and S4 with 4 lanes)

Validate Inputs and Selected Container & Register Step Started Automation

Automatically triggered at the beginning of the step, this automation invokes the `validate_flowcell_for_input_pools` and `validateSelectedContainer` scripts. These scripts validate the step inputs and the container selected by the user, as follows.

- Checks that the Flowcell Type field has been set to a valid value (SP, S1, S2, or S4) and that all inputs have the same value.
- Checks that the number of outputs matches the number of lanes on the selected flow cell type. If validation fails, an error message informs that the number of working pools does not match the number of lanes available on the flow cell.
  The following table defines the number of samples allowed:

Flow Cell Type	Number of Working Pools
SP	2
S1	2
S2	2
S4	4

- Checks that the container type selected on entry to the Placement screen matches the value in the Flowcell Type field. If validation fails, an error message displays.

```
script:validate_flowcell_for_input_pools \
-i {stepURI:v2} \
-u {username} \
-p {password} \
-l {compoundOutputFileLuid1} \
-validateSelectedContainer true \
```

- Registers the start time of the step by publishing messages to CLPA through the ICA LINK service:

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/common/step_started.groovy'
```

This automation is used only for CLPA support.

Validate Flowcell Barcode Automation

Automatically triggered on exit of the Placement screen, this automation validates the flow cell barcode scanned into the Clarity LIMS by the user, using the following logic:

```
if (input.::Flowcell Type:: == ::SP:: && !output.container.name.matches( ::[A-Z0-9]{5}DR[A-Z0-9]{2}:: ) ) {
fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: )
};
if (input.::Flowcell Type:: == ::S1:: && !output.container.name.matches( ::[A-Z0-9]{5}DR[A-Z0-9]{2}:: ) ) {
fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: )
};
if (input.::Flowcell Type:: == ::S2:: && !output.container.name.matches( ::[A-Z0-9]{5}DM[A-Z0-9]{2}:: ) ) {
fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: )
};
if (input.::Flowcell Type:: == ::S4:: && !output.container.name.matches( ::[A-Z0-9]{5}DS[A-Z0-9]{2}:: ) ) {
fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: )
};
```

- Copies the Flowcell Type and Loading Workflow Type field values from step inputs to outputs:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ;
output.::Loading Workflow Type:: = input.::Loading Workflow Type::
```

Validate Run Setup and Generate Sample Sheet Automation

The script definition portion of Validate Run Setup has been moved into the Validation Script custom field due to the 4000 character limitation.

- If modification is required to the Validate Run Setup script, perform the changes directly on Validation Script custom field default value.
- The script definition portion of Generate Sample Sheet is unchanged.

Automatically triggered by selecting a button on the Record Details screen, this automation completes the following actions:

- Copies the Flowcell Type from the step input to the Run Mode field (hidden):

```
step.::Run Mode:: = input.::Flowcell Type::
```

- Validates the parameters entered on the Record Details screen, which are used to set up the run and generate the sample sheet file. For more information, refer to the *Respond to Validation / Recipe Request Call from Instrument* section of *NovaSeq 6000 Run* on page 317.
  - Experiment Name can only contain alphanumeric, dash, or underscore characters. Spaces are not permitted.
  - When Workflow Type = No Index, both Index Read 1 and Index Read 2 must be zero.
  - When Workflow Type = Single Index, Index Read 1 must be greater than zero and Index Read 2 must be zero.
  - When Workflow Type = Dual Index, both Index Read 1 and Index Read 2 must be greater than zero.

```
if(!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) {
fail(::Experiment Name contains prohibited characters. Allowed characters
are: a-z, A-Z, 0-9, -, and _::)
};
if (step.::Workflow Type:: == ::No Index::) {
if (step.::Index Read 1:: != 0 || step.::Index Read 2:: != 0) {
fail(::Index Read 1 and Index Read 2 must be 0 if No Index is selected.::)
}
} else if (step.::Workflow Type:: == ::Single Index::) {
if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) {
fail(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if
Single Index is selected.::)
}
} else {
if (step.::Index Read 1:: == 0 || step.::Index Read 2:: == 0) {
```

```
fail(::Index Read 1 and Index Read 2 must be greater than 0 if Dual Index is
selected.::)
}
};
```

- Checks the Paired End and Read 2 Cycles field values.
  - If Paired End = True and Read 2 Cycles value is 0, an error displays.
  - If Paired End = False, Read 2 Cycles must be zero and UMI - Read 2 Length and UMI - Read 2 Start From Cycle must be empty.

```
if (step.::Paired End::.toBoolean()) {
if (step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) {
fail(::Read 1 Cycles and Read 2 Cycles must be greater than 0 if Paired End
is True.::)
}
} else {
if (step.::Read 2 Cycles:: != 0) {
fail(::Read 2 Cycles must be 0 if Paired End is False.::)
};
if (step.hasValue(::UMI - Read 2 Length::) || step.hasValue(::UMI - Read 2
Start From Cycle::)) {
fail(::UMI - Read 2 Length and UMI - Read 2 Start From Cycle cannot be
defined if Paired End is False.::)
}
};
```

- Checks the Flowcell field value.

  If Flowcell Type value is not SP, checks that the value of both Read 1 Cycles and Read 2 Cycles is 151 or less. If value is greater than 151, the automation generates an error message.

```
if (input.::Flowcell Type::!=::SP:: && step.::Read 1 Cycles:: > 151) {
fail(::Read 1 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
}
if (input.::Flowcell Type::!=::SP:: && step.::Read 2 Cycles:: > 151) {
fail(::Read 2 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
}
```

- Validates UMI Settings:
  - If UMI - Read 1 Length has value, UMI - Read 1 Start From Cycle must have value.
  - If UMI - Read 1 Length has no value, UMI - Read 1 Start From Cycle must not have value.
  - If UMI - Read 2 Length has value, UMI - Read 2 Start From Cycle must have value.
  - If UMI - Read 2 Length has no value, UMI - Read 2 Start From Cycle must not have value.
  - If UMI - Read 1 Length has not value, UMI - Read 2 Length must not have value.

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  - If UMI - Read 1 Start From Cycle has no value, UMI - Read 2 Start From Cycle must not have value.

```
if (step.hasValue(::UMI - Read 1 Length::) && !step.hasValue(::UMI - Read 1 Start From Cycle::)) {
fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 1 Length is greater than 0.::)
};
if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 1 Start From Cycle::)) {
fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 1 Start From Cycle is greater than 0.::)
};
if (step.hasValue(::UMI - Read 2 Length::) && !step.hasValue(::UMI - Read 2 Start From Cycle::)) {
fail(::UMI - Read 2 Start From Cycle must be greater than 0 if UMI - Read 2 Length is greater than 0.::)
};
if (!step.hasValue(::UMI - Read 2 Length::) && step.hasValue(::UMI - Read 2 Start From Cycle::)) {
fail(::UMI - Read 2 Length must be greater than 0 if UMI - Read 2 Start From Cycle is greater than 0.::)
};
if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 2 Length::)) {
fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 2 Length is greater than 0.::)
};
if (!step.hasValue(::UMI - Read 1 Start From Cycle::) && step.hasValue(::UMI - Read 2 Start From Cycle::)) {
fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 2 Start From Cycle is greater than 0.::)
};
```

- Validates Analysis Software Version to make sure it is a valid version string.

```
if (step.hasValue(::Analysis Software Version::) && !step.::Analysis Software Version::.matches(::^(\\d+)(\\.\\d+)*$::)) {
fail(::Analysis Software Version contains prohibited characters. Allowed characters are 0-9 and period. It shall start and end with numbers and separated by single period e.g. 3.8.4::)
};
```


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- Checks Override Cycles field value. This value can contain only Y, N, I, U, 0–9, and semicolon characters.

```
if (step.hasValue(::Override Cycles::) && !step.::Override Cycles::.matches(::
[YNIU0-9;]+::)) {
fail(::Override Cycles contains prohibited characters. Allowed characters are:
Y, N, I, U, 0-9 and ;. Example: N1Y150;I8;I7N1;Y141U10.::)
};
```

- Outputs the Settings Header in sample sheet when UMI - Read 1 Length or UMI - Read 2 Length have values.

```
step.::Settings Header:: = step.hasValue(::UMI - Read 1 Length::) ||
step.hasValue(::UMI - Read 2 Length::) ? ::[Settings]:: : ::::;
```

- Sets the next step for samples to ADVANCE, which advances to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8) step.

```
nextStep = ::ADVANCE::
```

- Generates the sample sheet and attaches it to the step. The sample sheet template depends on the selected value for Samplesheet Format and/or Reverse Complete Workflow. For more information, refer to *Sample Sheet Generation* on page 317.

```
&& if [ '{udf:Samplesheet Format}' == 'V2' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Samplesheet_v2.csv'; \
elif [ '{udf:Reverse Complement Workflow}' == 'true' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Reverse_Complement_Samplesheet.csv'; \
elif [ '{udf:Reverse Complement Workflow}' == 'false' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Samplesheet.csv'; \
else echo 'UDF Reverse Complement Workflow is empty or undefined.'; exit -1; fi
\
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/$templateFilename \
-o {compoundOutputFileLuid0}.csv \
-q true \
-destLIMSID {compoundOutputFileLuid0} \
-l {compoundOutputFileLuid1}"
```

Register Step Completed Automation

Registers the completion time of the step by publishing messages to CLPA through the ICA LINK service. This script is used only for CLPA support.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_
completed.groovy'
```

Master Step Fields

The following table lists the field configuration details defined on the Load to Flowcell (NovaSeq 6000 v3.8) step.

Table 112 Load to Flowcell (NovaSeq 6000 v3.8) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Analysis Software Version	Text		Not applicable
BaseSpace Sequence Hub Configuration	Text Dropdown	Required Field	Presets • Not Used • Run Monitoring Only • Run Monitoring and Storage
Custom Recipe Path	Text		Not applicable
Experiment Name	Text	Required Field	Not applicable
Index Read 1	Numeric Dropdown	Required Field Custom Entries	Range = 0–20 Decimal places displayed = 0 Presets • 0 • 6 • 8
Index Read 2	Numeric Dropdown	Required Field Custom Entries	Range = 0–20 Decimal places displayed = 0 Presets • 0 • 6 • 8
Library Tube Barcode	Text	Required Field	Not applicable

Field Name	Field Type	Options	Additional Options and Dropdown Items
Output Folder	Text	Required Field	Not applicable
Override Cycles	Text		Not applicable
Paired End	Text Dropdown	Required Field	Presets • True • False
Read 1 Cycles	Numeric Dropdown	Required Field Custom Entries	Range = 1–251 Decimal places displayed = 0 Presets • 251* • 151 • 101 • 51 *The value of 251 is only supported for SP flow cell type. For all other flow cell types, the maximum value is 151.
Read 2 Cycles	Numeric Dropdown	Required Field Custom Entries	Range = 0–251 Decimal places displayed = 0 Presets • 251* • 151 • 101 • 51 *The value of 251 is only supported for SP flow cell type. For all other flow cell types, the maximum value is 151.
Reverse Complement Workflow	Toggle Switch	Required Field	Default • Yes
Run Mode (not displayed in user interface)	Text Dropdown	Read Only	Presets • SP • S1 • S2 • S4

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Field Name	Field Type	Options	Additional Options and Dropdown Items
Samplesheet Format	Text Dropdown	Required Field	Presets • V1 (default) • V2
Settings Header (not displayed in user interface)	Text	Read Only	Not applicable
UMI - Read 1 Length	Numeric		Range = 1
UMI - Read 1 Start From Cycle	Numeric		Range = 1
UMI - Read 2 Length	Numeric		Range = 1
UMI - Read 2 Start From Cycle	Numeric		Range = 1
Use Custom Index Read 1 Primer	Toggle Switch		Default • None Set
Use Custom Read 1 Primer	Toggle Switch		Default • None Set
Use Custom Read 2 Primer	Toggle Switch		Default • None Set
Use Custom Recipe	Toggle Switch	Required Field	Default • No
Validation Script	Multiline Text	Required Field Read Only	Default value provided in the *Validation Script* on page 311 section following the table. Do not remove this field as it is used by Validate Run Setup and Generate Sample Sheet automation script.

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Field Name	Field Type	Options	Additional Options and Dropdown Items
Workflow	Text	Read Only	Default • GenerateFASTQ
Workflow Type	Text Dropdown	Required Field	Presets • No Index • Single Index • Dual Index • Custom

Validation Script

```
step.::Run Mode:: = input.::Flowcell Type::; if (!step.::Experiment
Name::.matches(::[a-zA-Z0-9-_]+::)) { fail(::Experiment Name contains prohibited
characters. Allowed characters are: a-z, A-Z, 0-9, -, and _::) }; if
(step.::Workflow Type:: == ::No Index::) { if (step.::Index Read 1:: != 0 ||
step.::Index Read 2:: != 0) { fail(::Index Read 1 and Index Read 2 must be 0 if
No Index is selected.::) } } else if (step.::Workflow Type:: == ::Single
Index::) { if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) { fail
(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if Single
Index is selected.::) } } else { if (step.::Index Read 1:: == 0 || step.::Index
Read 2:: == 0) { fail(::Index Read 1 and Index Read 2 must be greater than 0 if
Dual Index is selected.::) } }; if (step.::Paired End::.toBoolean()) { if
(step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) { fail(::Read 1
Cycles and Read 2 Cycles must be greater than 0 if Paired End is True.::) } }
else { if (step.::Read 2 Cycles:: != 0) { fail(::Read 2 Cycles must be 0 if
Paired End is False.::) }; if (step.hasValue(::UMI - Read 2 Length::) ||
step.hasValue(::UMI - Read 2 Start From Cycle::)) { fail(::UMI - Read 2 Length
and UMI - Read 2 Start From Cycle cannot be defined if Paired End is False.::) }
}; if (input.::Flowcell Type:: != ::SP:: && step.::Read 1 Cycles:: > 151) { fail
(::Read 1 Cycles must not be larger than 151 if it is not SPrime Flowcell::) };
if (input.::Flowcell Type:: != ::SP:: && step.::Read 2 Cycles:: > 151) { fail
(::Read 2 Cycles must not be larger than 151 if it is not SPrime Flowcell::) };
if (step.hasValue(::UMI - Read 1 Length::) && !step.hasValue(::UMI - Read 1
Start From Cycle::)) { fail(::UMI - Read 1 Start From Cycle must be greater than
0 if UMI - Read 1 Length is greater than 0.::) }; if (!step.hasValue(::UMI -
Read 1 Length::) && step.hasValue(::UMI - Read 1 Start From Cycle::)) { fail
(::UMI - Read 1 Length must be greater than 0 if UMI - Read 1 Start From Cycle
is greater than 0.::) }; if (step.hasValue(::UMI - Read 2 Length::) &&
!step.hasValue(::UMI - Read 2 Start From Cycle::)) { fail(::UMI - Read 2 Start
From Cycle must be greater than 0 if UMI - Read 2 Length is greater than 0.::)
```


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```
}; if (!step.hasValue(::UMI - Read 2 Length::) && step.hasValue(::UMI - Read 2
Start From Cycle::)) { fail(::UMI - Read 2 Length must be greater than 0 if UMI
- Read 2 Start From Cycle is greater than 0.::) }; if (!step.hasValue(::UMI -
Read 1 Length::) && step.hasValue(::UMI - Read 2 Length::)) { fail(::UMI - Read
1 Length must be greater than 0 if UMI - Read 2 Length is greater than 0.::) };
if (!step.hasValue(::UMI - Read 1 Start From Cycle::) && step.hasValue(::UMI -
Read 2 Start From Cycle::)) { fail(::UMI - Read 1 Start From Cycle must be
greater than 0 if UMI - Read 2 Start From Cycle is greater than 0.::) }; if
(step.hasValue(::Analysis Software Version::) && !step.::Analysis Software
Version::.matches(::^(\\d+)(\\.\\d+)*$::)) { fail(::Analysis Software Version
contains prohibited characters. Allowed characters are 0-9 and period. It shall
start and end with numbers and separated by single period e.g. 3.8.4::) }; if
(step.hasValue(::Override Cycles::) && !step.::Override Cycles::.matches(::
[YNIU0-9;]+::)) { fail(::Override Cycles contains prohibited characters. Allowed
characters are: Y, N, I, U, 0-9 and ;. Example: N1Y150;I8;I7N1;Y141U10.::) };
step.::Settings Header:: = step.hasValue(::UMI - Read 1 Length::) ||
step.hasValue(::UMI - Read 2 Length::) ? ::[Settings]:: : ::::; nextStep =
::ADVANCE::
```

Global Fields

The following table lists the global custom fields that are configured to display on the Load to Flowcell (NovaSeq 6000 v3.8) step.

Table 113 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Analyte in Derived Sample			
Flowcell Type	Text Dropdown	Required Field	Presets • SP • S1 • S2 • S4
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

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User Interaction

Refer to the Step 3: Load to Flowcell (NovaSeq 6000 v3.8) section in *NovaSeq 6000 Integration v3.6.0 Validation and Troubleshooting* on page 331.

Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8)

This final protocol contains one fully automated step, AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8), described in the following section.

> This step is fully automated. Do not add samples to the Ice Bucket or start the step manually. The sequencing service does not update samples correctly when they have been manually started.

Step 1: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8)

Step input: Library tube from NovaSeq Standard or flow cell from NovaSeq Xp protocol

Step output: Result file/measurement

In this step, pooled samples are sequenced on the NovaSeq instrument and the run metrics are recorded in Clarity LIMS.

Register Step Started & Register NovaSeq Run & Register NovaSeq Run Association & Register NovaSeq Run Started Automation

Automatically triggered at the beginning of the step, this automation completes the following actions:

- Registers the start time of the step
- Registers the NovaSeq 6000 sequencing run configuration
- Registers the association of the sequencing run with the actual samples by linking the Instrument Run ID with samples ID
- Registers the time that the sequencing run started

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-
product-analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/common/step_
started.groovy' && /opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/unified-product-analytics/automation/unified-
product-analytics-automation.jar script:executeUPAAutomationScript -i
{stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0} -s
'com/illumina/upa/scripts/novaseq6000/register_novaseq_run.groovy' &&
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/novaseq6000/novaseq_
```

```
associate_seqrun.groovy' && /opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/unified-product-analytics/automation/unified-
product-analytics-automation.jar script:executeUPAAutomationScript -i
{stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0} -s
'com/illumina/upa/scripts/novaseq6000/novaseq_seqrun_started.groovy'"
```

These actions are done by publishing messages to CLPA through the ICA LINK service. This automation is used only for CLPA support.

Set Next Steps Automation

By default, the Set Next Steps automation is disabled (set to Not Used) and the Sequencer API sets the next step for samples.

If necessary, override the default next step behavior used by the Sequencer API by performing the following steps:

1. Enable the automation and use it to set custom next step behavior and generate a log file. By default, the automation sets the next step to ADVANCE and the samples complete the protocol.
2. Configure the automation to trigger automatically on exit of the Record Details screen.
3. Disable or configure auto-complete behavior by setting the values of the run.autoComplete and autoCompleteOnlyAtSuccess properties. For details, refer to *Configuring autoComplete Properties* on page 329.

Update Lane Number Automation

This automation triggers automatically upon entry to the Record Details screen.

Do not disable or modify this automation to ensure the Lane Number displays properly.

Register NovaSeq RunCompleted & Register Step Completed

Automatically triggered upon exiting the step, this automation registers the sequencing run completion time, run status, and run metrics.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-
product-analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/novaseq6000/novaseq_
seqrun_completed.groovy' && /opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/unified-product-analytics/automation/unified-
product-analytics-automation.jar script:executeUPAAutomationScript -i
{stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0} -s
'com/illumina/upa/scripts/common/step_completed.groovy'"
```

These actions are done by publishing messages to CLPA through the ICA LINK service. This automation is used only for CLPA support.

Master Step Fields

The following fields are configured on the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8) step in Clarity LIMS:

- Current Cycle
- Current Read
- Firmware Version
- Flow Cell Expiration Date
- Flow Cell ID
- Flow Cell Lot Number
- Flow Cell Mode
- Flow Cell Part Number
- Flow Cell Side
- Instrument Control Software Version
- Instrument ID
- Instrument Type
- Lane Counter
- Loading Workflow Type
- Output Folder
- RTA Version
- Run Completion Date
- Run ID
- Run Status
- Sequencing Log

Global Fields

The following fields are used to capture the run metrics in Clarity LIMS:

- % Aligned R1
- % Aligned R2
- % Bases >=Q30 R1
- % Bases >=Q30 R2

- % Error Rate R1
- % Error Rate R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- %PF R1
- %PF R2
- Cluster Density (K/mm^2) R1
- Cluster Density (K/mm^2) R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- Reads PF (M) R1
- Reads PF (M) R2
- Yield PF (Gb) R1
- Yield PF (Gb) R2

Note the following details:

- Values are aggregated across all lanes. Some values (eg, Yield PF (Gb) R1) are summed while others are averaged.
- The names listed previously are the default global custom field names installed with the NovaSeq Integration v3.8 configuration provided in the Illumina Preset Protocols (IPP) v2.6 or later.
- All global configuration fields are configured on the Container entity.
- All field names are configurable through the Custom Fields screen in the Global Fields tab.
- If field names are changed in Clarity LIMS, they must also be changed through the `run.metricUdfNames` properties file in the `application.yml` file for the Sequencer API. For more information, refer to the *application.yml Properties* section in *Components Installed* on page 322. All fields are configured to be visible in the Sample Details table on the Record Details screen for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8) step.

These global custom fields are configured on the Container entity, visible on each input, but they should not be confused with per-lane metrics.

All run metrics tracked in Clarity LIMS are overall metrics for the run. There are currently no per-lane metrics provided by NovaSeq Control Software.

Sample Sheet Generation

The sample sheet is generated on the step before the run. This step is Dilute and Denature (NovaSeq 6000 v3.8) in the NovaSeq Standard protocol or Load to Flowcell (NovaSeq 6000 v3.8) in the NovaSeq Xp protocol. This step places samples on the library tube or flow cell that are loaded in the NovaSeq 6000 instrument.

In the default configuration, the Validate Run Setup and Generate Sample Sheet automation generates one CSV format sample sheet file. The bcl2fastq v2.20 downstream analysis uses this file.

The sample sheet is uploaded to the NVCS via the `/Illumina/Sequencer/v2/sequencing-run/files` endpoint to the Sequencer API. The file endpoint allows for a file to be downloaded from the Clarity LIMS using OAuth (instead of Basic Authentication), which is required for the NovaSeq 6000 instrument.

The run recipe response sets the sample sheet URL to the link to download the file from this endpoint and sets the sampleSheetRequiresOAuth value to true.

Sample Sheet Content

The following master step fields control the sample sheet content. These fields display in the Step Details area of the Record Details screen.

- Experiment Name
- Read 1 Cycles
- Read 2 Cycles
- Workflow
- Reverse Complement Workflow
- Samplesheet Format

Depending on Samplesheet Format field value selection, a v1 or v2 sample sheet is generated. For detailed information on the following items, refer to the *Bcl2fastq2 Sample Sheet Generation* section of *Illumina Instrument Sample Sheets (NGS v5.17 and later)*:

- Sample sheet generation script parameters and usage
- Sample sheet data and configuration options
- Enabling unique FASTQ file names per sequencing run

NovaSeq 6000 Run

The following steps outline the sequence of events that occurs when a flow cell is loaded onto the NovaSeq 6000 instrument.

User Authentication and Login

User authentication and login to the NovaSeq instrument is achieved via the getSequencerLoginURL endpoint (found at `/Illumina/Sequencer/v2/sequencing-run/login`). This endpoint returns the URL that the instrument uses to get an access token.

1. The Sequencer API service provides the NovaSeq Control Software (NVCS) with a URL for the Clarity LIMS login screen and the NVCS displays this screen to the user. Log in to authorize the NVCS to use your account automatically to access Clarity LIMS.
2. Enter credentials:
   - Username and password are passed to the Clarity LIMS server to make sure that the access is valid.
   - By default, the Sequencer API service tries to contact the Clarity LIMS at `http://localhost:9080/clarity/`. The installation script sets this URL, but it can be configured with the clarity.url property in the `application.yml` file. For more information, refer to the *application.yml Properties* section of *Components Installed* on page 322.
3. The user is redirected to the Sequencer login page at `/Illumina/Sequencer/v2/sequencer_login`.
   - If authentication is successful, the screen displays a success message. The Clarity LIMS server provides the OAuth token and username by returning them in redirect to `/Illumina/Sequencer/v2/sequencer_login/Success`. This redirect does not reload the page, but allows the NovaSeq to continue with run setup.
   - If authentication fails, the user is left on the login screen, and a warning message informs them of the failure. A background redirect to `/Illumina/Sequencer/v2/sequencer_login/Error` informs the NovaSeq 6000 of the failure.

Respond to Validation / Recipe Request Call from Instrument

1. The `sequencing-run/recipe/novaseq` endpoint consumes a POST request with a LibraryContainerId, FlowCellId, and reagents information.
   - LibraryContainerId, FlowCellId, and reagent information is provided by the NovaSeq 6000.
   - LibraryContainerId and FlowCellId can match either the library tube ID or flow cell ID of the corresponding containers in Clarity LIMS.
   - If there are no containers or multiple containers, an error is generated.
2. All populated containers whose name matches either the LibraryContainerId or FlowCellId are validated as follows.
   - All samples in the container must be queued for a sequencing run step.
   - All samples must be queued for only one sequencing run step.
   - All samples must be queued for the same sequencing run step.

> Configure the steps that are considered sequencing run steps in the novaseq.sequenceStepNames property in the `application.yml` file. The property is a list and each list entry must be the exact name of a sequencing step, not the name of the underlying master step/process type. For more information, refer to the *application.yml Properties* section of *Components Installed* on page 322.

3. After a single container has passed validation, the run setup information (run recipe request) is returned to the NVCS via the API. Then, the run recipe is generated.
   - The run recipe information is read from fields configured on the parent process/master step of the samples in the container. The workflow must include recipe definition in that step.
   - By default, the parent process/master step is the Dilute and Denature (NovaSeq 6000 v3.8) or Load to Flowcell (NovaSeq 6000 v3.8) step, depending on workflow.
   - The default field values match the names of the fields in the NovaSeq v3.8 workflow configuration included in IPP. The field names are configurable through the recipe.udfNames.* properties. Each of these properties corresponds to an entry in the recipe. The value of a property defines the name of the field that is used to populate that entry in the recipe.
   - It is assumed that this parent process/master step removes any illegal characters from the Experiment Name field.
   - The Clarity LIMS download link to the sample sheet created on the run setup step is used for the SampleSheet value in the recipe. The name of the output of the sample sheet on the setup step can be configured through the recipe.sampleSheet.outputName property (Sample Sheet by default) in the `application.yml` file. For more information, refer to the *application.yml Properties* section of *Components Installed* on page 322. If no matching output is found, or no file was attached to the output, then the value of the recipe.sampleSheet.notAvailableValue property (Not Available by default) is used in the recipe.
4. After the recipe is generated, it is returned as a *.json file in the response, in the form defined by the Swagger definition for the API.

Run Recipe Content

The sample sheet content and the fields configured on the Dilute and Denature (NovaSeq 6000 v3.8) and Load to Flowcell (NovaSeq 6000 v3.8) steps control the content of the run recipe.

The following example shows the run recipe content:

```
{
"run_name": "NVCS16027_SPrime_2x250",
"run_mode": "S4",
"workflow_type": "DualIndex",
"librarytube_id": "NV0025867-LIB",
"flowcell_id": "",
"sample_loading_type": "NovaSeqStandard",
```


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```
"rehyb": false,
"paired_end": true,
"read1": 151,
"read2": 151,
"index_read1": 8,
"index_read2": 8,
"output_folder": "\\\\network_path\\run_data",
"samplesheet": "<path to sample sheet on LIMS server>",
"require_samplesheet_authentication": true,
"usecustomrecipe": false,
"customRecipe": null,
"use_basespace": false,
"basespace_mode": null,
"use_custom_read1_primer": false,
"use_custom_read2_primer": false,
"use_custom_index_read1_primer": false
}
```


Respond to Sequencing Started Call from Instrument

1. Matching container for the run (library tube or flow cell) is found, and contents are validated to be queued for the correct step.

   If no matching container is found, or if the samples in the container are incorrectly queued, an error occurs and the step is not started. This validation is also done during the run recipe request immediately prior, so this error case should not be encountered.

2. Reagent kits that are configured for the Sequencer API are checked against the configuration for the step to be run:
   - If a kit is configured for the API but does not exist in Clarity LIMS, it is created.
   - If a kit exists, but is not enabled on the step to be run, the step configuration is updated to enable it.
     - If a kit that is to be enabled is Archived, it is set to Active.
     - If a kit that is to be enabled is in Pending status, it is set to Active.
   - If any kits enabled on the step are not configured for the Sequencer API, a warning is logged. The API cannot complete the step without user intervention.
   - If any kits provided in the recipe request are not configured for the Sequencer API, they are ignored. A warning is logged during lot tracking.
3. The type of flow cell being used in the run determines the number of replicates for the step.

- For Standard mode, this information is based on the number of lanes in the flow cell. This information is stored as part of the Sequencer API configuration.
- For XP mode, there is always one replicate, because there is already one input tracked for each lane.

4. The automated sequencing step is started.
5. Reagent lots are attached to the step. The Flow Cell and Library Tube reagents in the request are ignored. Information from these reagents is tracked in Clarity LIMS as containers and custom fields. Any other reagents in the request are handled as follows.
   - If the reagent is not configured to be tracked, a warning is logged, and the reagent is ignored.
   - Existing reagent lots are looked up by kit name (determined by Sequencer API configuration), name (the serial number in the request), and lot number (the lot number in the request).
   - If one or more matching reagent lots exist in Clarity LIMS, the newest one is used.
   - If the expiry date does not match the expiry in the request, or if the status is not set to Active, the lot is updated to have the correct expiry date and an Active status.

   Otherwise a new lot is created with the following configuration:
   - Reagent Kit—Matching kit as determined from Sequencer API configuration and 2.
   - Name—Serial number in request
   - Lot Number—Lot number in request
   - Expiry—Expiry date in request
   - Status—Active

   If any reagents are expected to be in the request but are not, these reagents are logged. This action prevents the step from being completed without user intervention.
6. Master step custom fields are updated with the run information parsed from the request. For more information, refer to *Respond to Record InterOps Metrics Call from Instrument* on page 321.
7. Any important events during this request are logged into the Sequencer API log file and in the Sequencing Log multiline global custom field on the step (assuming the step was started successfully). For more information, refer to *Respond to Record InterOps Metrics Call from Instrument* on page 321.

Respond to Record InterOps Metrics Call from Instrument

When the metrics endpoint receives a POST, it completes the following actions:

- Looks for a matching library tube or flow cell in Clarity LIMS.
- Find the in-progress sequencing step for that container.

When the step is found, the run metrics in the request are written to container global custom fields in Clarity LIMS.

Respond to Sequencing Completed Call from Instrument

If the sequencing run is successful, after the metrics are recorded, the API call for Sequencing Completed is received. Information is matched by Sequencing Container and the following occurs in Clarity LIMS:

1. The Current Cycle, Current Read, and Status master step custom fields are updated.
2. The multiline Sequencing Log master step field is updated.
3. A success message is recorded in the log file at the info level:

```
Sequencing run with Run ID {runId} of Step '{step.name}' ({step.limsid})
completed successfully.
```

4. The Clarity LIMS waits for any automations that are triggered automatically as it advances the step.
5. By default, the Clarity LIMS assigns the next steps on the outputs to move them on to the first configured next step. If there are no next steps, the protocol is completed.

Respond to Sequencing Errored Call from Instrument

If the sequencing run is unsuccessful, the API call for Sequencing Error is received. Information is matched by Sequencing Container and the following occurs in Clarity LIMS:

1. The Current Cycle, Current Read, and Status master step custom fields are updated.
2. The multiline Sequencing Log master step field is updated.
3. A failure message is recorded in the log file at the error level:

```
Step '${Step.name}' (${Step.limsid}) failed with Run ID ${runId}.
```

4. The step is left at the Record Details screen.

Respond to Sequencing RunEndedByUser Call from Sequencer

If you end a sequencing run, the API call for Sequencing RunEndedByUser is received. Information is matched by Sequencing Container and the following occurs in Clarity LIMS:

1. The Current Cycle, Current Read, and Status master fields are updated.
2. The multiline Sequencing Log master step field is updated.
3. The following message is recorded in the log file, at the warning level:

```
"Sequencing run with Run ID ${status.runInfo.runId} of Step
'${step.configuration.name}' (${step.limsid}) aborted by user
${status.runInfo.userName}."
```

4. The step is left at the Record Details screen.

Components Installed

The following sections describe the components (files, properties, reagent categories / label groups, reagent kits, and containers) that are installed by default as part of this integration.

Illumina NovaSeq 6000 Integration v3.6.0 is distributed as the `BaseSpaceLIMS-sequencer-api` RPM package. This RPM package must be installed on the Clarity LIMS server.

The `BaseSpaceLIMS-sequencer-api` RPM installs the following items:

- Sequencer API WAR file
- `application.yml` configuration file
- Two configuration scripts: `configure_sequencer_api_proxy.sh` and `configure_sequencer_api_application.sh`

The NovaSeq 6000 v3 configuration is delivered through Illumina Preset Protocols (IPP) v2.6, which requires installation of the NGS Extensions Package v5.23.0 or later.

- If the NGS Extensions Package is not already installed, or if a version earlier than v5.23.0 is installed, the latest version is installed by default with the NovaSeq 6000 integration. For more information, refer to *NovaSeq 6000 Integration v3.6 Release Notes* on page 368.
- If NGS Extensions Package v5.23.0 or later is already installed, the upgrade is not forced or required.

NovaSeq 6000 Integration RPM Components

The following table lists the components installed by the RPM package.

Files Installed	Location and Description
`Illumina#Sequencer#v2.war`	`/opt/gls/clarity/tomcat/current/webapps` War file for Sequencer API application.
`configure_sequencer_api_application.sh`	`/opt/gls/clarity/config/` Script that configures the Sequencer API application through its external `application.yml` file.
`configure_sequencer_api_env.sh`	`/opt/gls/clarity/config/` Script that configures the Clarity LIMS Tomcat configuration to include Secret Util settings.
`configure_sequencer_api_proxy.sh`	`/opt/gls/clarity/config/` Script that configures the proxy to allow communication with the Sequencer API application.
`sequencer-api.conf`	`/etc/httpd/clarity/` Proxy setting to communicate with the Clarity LIMS.

application.yml Properties

The `application.yml` file is at `opt/gls/clarity/extensions/sequencer-api/`.

- All properties are configured automatically during installation, but they can also be configured by editing the `application.yml` file and then restarting Tomcat.
- Properties in the file represent all custom field names, both for recipe and run information.
- For all custom fields, the names (values in this file) can be changed. However, the property names cannot be changed, removed, or added to. For example, Loading Workflow Type can be changed, but recipe.udfNames.sampleLoadingType cannot.

Table 114 application.yml Properties Installed

Property	Description
spring.profiles.active	Tells the application that it is running deployed in Tomcat. Default (do not change) • tomcat
clarity.url	Base URL that the Sequencer API service uses to contact the Clarity LIMS. The installation script (`configure_sequencer_api_application`) prompts for this URL.
clarity.username	Username to be used when communicating with Clarity LIMS.
security.signing-key	Private key that is used when signing/validating OAuth tokens. Changing this key invalidates any issued tokens.
security.token-expiry	Specifies (in hours) the expiry period for login tokens issued by the Sequencer API. Default • 88

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Property	Description
novaseq.sequenceStepNames	List of NovaSeq sequencing run step names for which the integration can find samples queued. • Names must be an exact match to the name of the step in Clarity LIMS (not the master step/process type). • Each line must be indented to the same point and start with a dash (-). Default - "AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8)" - "AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1)"

novaseq.flowcells	List of supported NovaSeq flow cell types.
	• Type names exactly match the values used by the NVCS run status requests.
	• To add more types, provide both type and lanes properties, indented to the same point and starting with a dash (-).
	Default
	`- type: "SP"`
	`lanes: 2`
	`- type: "S1"`
	`lanes: 2`
	`- type: "S2"`
	`lanes: 2`
	`- type: "S4"`
	`lanes: 4`

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Property	Description

novaseq.reagents	List of supported reagent kits to be tracked on sequencing steps.
	• **- lotName**—The name of the lot as it appears in the run started request.
	• - kitName—The name of the corresponding reagent kit in Clarity LIMS that the lot is tracked as.
	• The name can differ in the NVCS request versus the Clarity LIMS configuration.
	Default
	`- lotName: "Buffer"`
	`kitName: "Buffer Cartridge"`
	`- lotName: "Cluster"`
	`kitName: "Cluster Cartridge"`
	`- lotName: "SBS"`
	`kitName: "SBS Cartridge"`

recipe.udfNames	Configures the names of the fields used in the run recipe. For more information, refer to the *Run Recipe Contents* section of *NovaSeq 6000 Run* on page 317. • Each value must be enclosed in quotes (eg, recipe.udfNames.runMode "Run Mode"). • Value provided for sampleLoadingType must match the value provided in the run configuration (eg, recipe.udfNames.sampleLoadingType "Loading Workflow Type").
recipe.sampleSheet.outputName recipe.sampleSheet.notAvailableValue	Configures the name of the sample sheet file placeholder on the step where run recipe information is populated, and the property value to use when the file is not found.

Property	Description
run.stepUdfNames	Configures the master step field names to be used when recording run results. For more information, refer to the *Master Step Fields* section of *Step 1: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7)* in *Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8)* on page 313. • Each value must be enclosed in quotes (eg, run.stepUdfNames.runID "Run ID") • Value provided for sampleLoadingType must match the value provided in the run configuration (eg, run.stepUdfNames.sampleLoadingType "Loading Workflow Type"). It is expected that the run step field and the field on the input derived sample share a name.
run.metricUdfNames	Configures the result file custom fields/measurement global field names to be used when recording run results. These fields capture the run data. For more information, refer to the *Global Fields* section of *Step 1: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7)* in *Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8)* on page 313.
run.autoComplete	Determines whether the sequencing run step must be completed automatically after parsing the run metrics on a run along with run.autoCompleteOnlyAtSuccess. For more information, refer to *Configuring autoComplete Properties* on page 329. Default • true

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Property	Description
run.autoCompleteOnlyAtSuccess	Determines which of the following options that the sequencing run step must be: • Autocompleted when the run status is RunCompletedSuccessfully (if autoCompleteOnlyAtSuccess is true) • Autocompleted regardless of the run status (if autoCompleteOnlyAtSuccess is false). For more information, refer to *Configuring autoComplete Properties* on page 329. Default • true

Reagent Categories/Label Groups

- TruSeq HT Adapters v2 (D7-D5)

Reagent Kits

- Buffer Cartridge
- Cluster Cartridge
- DPX1
- DPX2
- DPX3
- NaOH
- Resuspension Buffer (RSB)
- SBS Cartridge
- Tris HCl

Container Types

- Library Tube
- SP
- S1
- S2
- S4

This integration supports the following items:

- Library tube with barcode provided in the format [A-Z]{2}[0-9]{7}-[A-Z]{3} (eg, AB1234567-XYZ)
- SP, S1, S2, or S4 flow cell with barcode provided in one of the following formats:
  - SP and S1 flow cell: [A-Z0-9]{5}DR[A-Z0-9]{2}
  - S2 flow cell: [A-Z0-9]{5}DM[A-Z0-9]{2}
  - S4 flow cell: [A-Z0-9]{5}DS[A-Z0-9]{2}
  - Example: H1991DMXX

NVCS Configuration

For details on configuring NVCS for integration with Clarity LIMS, contact the Clarity LIMS Support team.

Routing Script Requirements

The requirements for the routing script functionality are as follows.

1. On the steps that use the routing script (Define Run Format (NovaSeq 6000 v3.8) and Dilute and Denature (NovaSeq 6000 v3.8)), the Next Step for all samples must be set to Remove from workflow. A script sets this value. The value must not change in the Assign Next Steps screen.
2. In the Next Steps section of the protocol configuration screen, the method of assigning the next step must be set to Automation for the last step of the protocol.

Configuring autoComplete Properties

The `application.yml` configuration file contains the following properties:

- run.autoComplete
- run.autoCompleteOnlyAtSuccess

These properties determine the conditions under which the last step of the NovaSeq 6000 workflow (AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8)) is automatically completed when the run status is "RunCompletedSuccessfully" (default behavior) or regardless of the run status.

By default, both properties are set to true and the step only completes when the run is successful. If the run fails or is aborted, manually complete the step in Clarity LIMS.

For the step to autoComplete regardless of the result of the sequencing run, change the autoCompleteOnlyAtSuccess property value to false.

Enabling/Disabling autoComplete Properties

1. Open the `application.yml` file at `/opt/gls/clarity/extensions/sequencer-api/application.yml`:

```
vi /opt/gls/clarity/extensions/sequencer-api/application.yml
```

2. Edit the value of the autoCompleteOnlyAtSuccessproperty as required.
3. Save the file.

The following table shows how the combined value of the run.autoComplete and run.autoCompleteOnlyAtSuccess properties affects the autoComplete behavior of the sequencing step.

Table 115 run.autoComplete and run.autoCompleteOnlyAtSuccess Value Matrix

run.autoComplete Property Value	run.autoCompleteOnlyAtSuccess Property Value	Outcome
true	true	• Step automatically completes only if sequencing run is successful. • If run is not successful, step does not automatically complete, and the run details are recorded in Clarity LIMS.
true	false	• Step automatically completes, regardless of the run status.
false	true	• Step does not automatically complete. • Run details are recorded in Clarity LIMS.
false	false	

Rules and Constraints

- The workflow configuration contains several validation checks. To make sure that the calculations work properly, it is important that you do not disable any of this validation logic. The validation checks determine the following information:
  - Which samples, and how many, can enter each step together.
  - Which samples, and how many, can be pooled together.
- The library tube ID must be unique. There must not be multiple library tube containers in the system with the same name.
- Reagent labels (indexes) must be unique.
- Only controls are permitted as unindexed samples; all other unindexed samples and pools are not permitted.
- For sample sheet generation constraints, refer to the *Bcl2fastq2 Sample Sheet Generation* section in *Illumina Instrument Sample Sheets (NGS v5.17 & later)*.

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- Do not manually start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8) step. This step is a fully automated and the sequencing service does not update samples correctly if they have been manually started.
- For the automated run to start successfully, Validate Run Setup and Generate Sample Sheet must be selected.

NovaSeq 6000 Integration v3.6.0 Validation and Troubleshooting

This section explains how to validate the installation of the Illumina NovaSeq 6000 Integration Package v3.6.0.

The validation process involves the following items:

- Running samples through the Library Prep Validation workflow.
  - The workflow contains a single-step protocol that models the library prep required to produce normalized libraries. At the end of the step, the normalized libraries advance to the workflow selected.
- Running normalized libraries through the NovaSeq 6000 v3.8 workflow validates the following items:
  - Successful routing of samples from the Run Format (NovaSeq 6000 v3.8) step to the NovaSeq Standard (NovaSeq 6000 v3.8) or NovaSeq Xp (NovaSeq 6000 v3.8) step.
  - Automated generation of v1 sample sheet for use with bcl2fastq2 v2.20 analysis software or v2 sample sheet. This file automatically uploads to the sequencing system via the Sequencer API.
  - Automatic validation of run setup information. Information is uploaded to the NovaSeq Control Software (NVCS) via the Sequencer API and is used to create the run recipe and initiate the run.
  - Automated tracking of the NovaSeq sequencing run and parsing of run statistics into Clarity LIMS, via the Sequencer API.

The validation steps assume that the Illumina NovaSeq 6000 Integration Package v3.6.0 is installed and you have imported the default Clarity LIMS configuration.

Activate Workflow, Create Project, Add and Assign Samples

The following steps set up Clarity LIMS in preparation for running samples through the Library Prep Validation and NovaSeq 6000 v3.8 workflows.

1. On the Configuration tab, under Workflows, activate both the Library Prep Validation and NovaSeq 6000 v3.8 workflows.
   - Library Prep Validation v2.3.1 is available with Illumina Preset Protocols (IPP) v2.6.
2. On the Projects and Samples screen, create a project and add samples to it.
3. Assign the samples to the Library Prep Validation workflow.

Library Prep Protocol: Library Prep Validation v2.3.1

This single-step protocol models the library prep required to produce normalized libraries that are ready for the NovaSeq 6000 v3.8 workflow.

Run Library Prep Validation v2.3.1

1. In Lab View, locate the Library Prep Validation v2.3.1 protocol.
   Samples are queued for the Library Prep Validation v2.3.1 step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Placement screen, complete the following actions:
   a. In the Placed Samples area on the right, in the Container field, scan or enter the barcode of the destination container.
   b. Select the samples from the Samples to be Placed area on the left side, and drag them to the container wells on the right side.
   c. Select Add Labels.
5. On the Add Labels screen, the Labels to Add list is populated with labels from the TruSeq HT Adapters v2 (D7-D5) label group. The group is the only label group configured in the integration and is used by default in this step.
   a. Select labels on the left side, and drag them to the container on the right side to assign them to placed samples.
   b. Select Record Details.
6. On the Record Details screen, complete the following actions:
   a. In the Sample Details table, enter values for Normalized Molarity (nM). Type values individually or copy and paste to enter multiple values from a spreadsheet.
   b. The Sequencing Instrument column indicates the instrument to be used for the sequencing run. Select NovaSeq 3.0.
   c. Populate the fields in this column using one of the following methods:
      - To populate fields individually for each sample, select from the drop-down list in each row.
      - To populate all fields at one time, copy and paste from a spreadsheet.

   Alternatively, select inside the table and press Ctrl + A to select all rows. At the top of the table, select Sequencing Instrument and then select NovaSeq 3.0 from the adjacent drop-down list. Select Apply.

   If working with many samples, copy and paste to insert multiple values from a spreadsheet as follows.

   a. Select Copy Samples to copy the sample information from Clarity LIMS to the Clipboard.
   b. Open a blank Excel document and paste the contents of the clipboard into the spreadsheet.

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c. In the spreadsheet, enter the Normalized Molarity and Sequencing Instrument values. Select the entire spreadsheet including the headers (Ctrl + A), and copy (Ctrl + C) the information to the Clipboard.

d. Within Clarity LIMS, select the Paste Samples button. Paste the contents of the clipboard (Ctrl + V) into the Update Table window and then select Update to paste the contents into the Sample Details table.

7. Select Next Steps.
   Triggers the Set Next Steps automation, which sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step.
8. On the Assign Next Steps screen in the Samples table, the Next Step value for all samples is prepopulated with Remove from workflow.

   Do not change this value. If Next Step is not set to Remove from workflow, the routing script does not route samples correctly.

9. Select Finish Step.
   On exit from the step, the Routing Script automation is triggered. This automation assigns samples to the first step of the NovaSeq 6000 v3.8 workflow, Define Run Format (NovaSeq 6000 v3.8). This step is the only one in Protocol 1: Run Format (NovaSeq 6000 v3.8).

Protocol 1: Run Format (NovaSeq 6000 v3.8)

This protocol includes a single step, Define Run Format (NovaSeq 6000 v3.8). The step allows for the assignment of per sample values for Loading Workflow Type, Normalized Molarity, Flowcell Type, and Final Loading Concentration (pM). At the end of the step, samples are routed to the NovaSeq Standard or NovaSeq Xp protocol, according to the selected Loading Workflow Type.

Step 1: Define Run Format (NovaSeq 6000 v3.8)

1. In Lab View, locate the Run Format (NovaSeq 6000 v3.8) protocol. The samples are queued for the Define Run Format (NovaSeq 6000 v3.8) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Record Details screen in the Sample Details table, populate the following fields (values can vary across samples):
   - Loading Workflow Type—Select NovaSeq Standard or NovaSeq Xp from the drop-down list.
   - Flowcell Type—Select SP, S1, S2, or S4.
   - Final Loading Concentration (pM)—Select from the two preset options: 225 (for PCR-free workflows), 400 (for Nano workflows), or enter a different value.

- Normalized Molarity (nM)—These values are copied over from the previous step. If this column is not populated during library prep, enter the values here.

5. Select Next Steps, which triggers the Set Next Steps automation and the following actions:
   - Sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step.
   - Calculates the Minimum Molarity.
   - Checks Normalized Molarity value. For samples with no Normalized Molarity value (an empty value not including 0), generates an error message notifying that the field cannot be empty.
   - Compares each sample Normalized Molarity value with the Minimum Molarity value.
6. On the Assign Next Steps screen:
   - In the Sample Details table, the Next Step for all samples is prepopulated with Remove from workflow, regardless of the Loading Workflow Type.

     Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not routed samples correctly.

   - For samples whose Normalized Molarity value is lower than the Minimum Molarity value, the Loading Workflow Type is set to Remove from workflow. Also, a message is recorded in the Warning field for the sample.
     At this point, there are two options:
     - Return to the Record Details screen and adjust the Normalized Molarity value so that it equals or exceeds the Minimum Molarity value. The Loading Workflow Type must be set to NovaSeq Standard or NovaSeq Xp, as applicable.
     - Complete the protocol without correcting the Normalized Molarity value. In this case, the samples in question are removed from the Clarity LIMS workflow.
7. Select Finish Step. The Routing Script & Register Step Completed automation is triggered:
   - Samples whose Loading Workflow Type is set to Remove from workflow are removed from the Clarity LIMS workflow. (Occurs when the Normalized Molarity value is lower than the Minimum Molarity.)
   - Samples whose Loading Workflow Type is set to NovaSeq Standard are routed to the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.8) step. This step is the first one in the NovaSeq Standard protocol.
   - Samples whose Loading Workflow Type is set to NovaSeq Xp are routed to the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.8) step. This step is the first one in the NovaSeq Xp protocol.

Protocol 2: NovaSeq Standard (NovaSeq 6000 v3.8)

In this protocol, samples are pooled and added to the library tube in preparation for the NovaSeq run.

Step 1: Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.8)

1. In Lab View, locate the NovaSeq Standard (NovaSeq 6000 v3.8) protocol. Samples for the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.8) step are queued.
2. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Pooling screen:
   a. Create a pool by dragging samples into the Pool Creator.

      Only create one pool.

   b. Enter a name for the pool or accept the default name (Pool #1).
   c. Select Record Details.
      After exiting from the Pooling screen, the Validate Inputs Flowcell Type and Single Pool automation is triggered. The automation verifies the following items:
      - All samples in the pool have been assigned the same Flowcell Type.
      - Only one pool has been created.
5. On the Record Details screen in the Step Details area, complete the two required fields and one optional field, as needed:
   - Number of Flowcells to Sequence—Used in volume calculations, to make sure that the volumes are sufficient for the number of times the pool is sequenced.
   - Minimum Per Sample Volume (µl)—Used to calculate how much of each sample is included in the pool. The field is prepopulated with the configured default value 5 µl, but can be edited.
   - % PhiX (2.5 nM) Spike-In—Used to calculate the volume of PhiX v3 control included in the pool for the given percentage of spike-in. The field is optional and can be edited.

     Assuming the default Minimum Per Sample Volume (µl) value of 5, for a given batch:
     - If the smallest Per Sample Volume (µl) value is < 5, Clarity LIMS automatically assigns a value of 5 to the Adjusted Per Sample Volume (µl) field.
     - Clarity LIMS changes the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.
6. In the Sample Details table, select the pool icon to view details on the pool composition.
7. Select Calculate Volumes to trigger the Calculate Volumes automation. This automation performs calculations based on the selected Flowcell Type.
   - Calculates Bulk Pool Volume (µl) value.
   - Sets the value of the Bulk Pool Volume (µl) field.

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- Calculates the PhiX Volume (µl) value when % PhiX (2.5 nM) Spike-In has value.
- Calculates the Per Sample Volume (µl) value to be added to the pool.
- Calculates the Total Sample Volume (µl) value.
- If the Total Sample Volume is less than the Bulk Pool Volume, selects the RSB Volume (µl) value.
- Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
- Populates the Volume of Pool to Denature (µl), NaOH Volume (µl) and Tris-HCl Volume (µl) columns of the Sample Details table. The script sets the values, and is not editable.
- The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains volume information about the pool and the individual samples that it contains. Select on the file to download it and then open it in Excel.

8. Select Next Steps to trigger the Set Next Steps automation. This automation sets the next step for samples to ADVANCE and advances them to the next step in the protocol, Dilute, and Denature (NovaSeq 6000 v3.8).
   On the Assign Next Steps screen, the next step for samples is set to the next step in the workflow: Dilute and Denature (NovaSeq 6000 v3.8).
9. Select Finish Step.
   At the end of this step, the pool of samples automatically advances to the Dilute and Denature (NovaSeq 6000 v3.8) step.

Step 2: Dilute and Denature (NovaSeq 6000 v3.8)

1. In Lab View, locate the NovaSeq Standard (NovaSeq 6000 v3.8) protocol. The pool of samples queued for the Dilute and Denature (NovaSeq 6000 v3.8) step are listed.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work. The Validate Single Input & Register Step Started automation is triggered. This automation verifies that only one container is input for the step.
4. On the Placement screen:
   a. Drag the pool into the library tube in the Placed Samples area.
   b. Scan or type the barcode of the library tube into the Library Tube field.
   c. Select Record Details.
   After exiting the Placement screen, the Validate Library Tube Barcode automation makes sure that the library tube barcode conforms to the barcode mask [A-Z]{2}[0-9]{7}-[A-Z]{3}. If it does not, an error message displays. The automation also copies the Flowcell Type and Loading Workflow Type values from step inputs to outputs.
5. On the Record Details screen, the Reagent Lot Tracking section lets you tracks the NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. Add and activate lots for these reagents.

To add and activate reagent lots:

a. In a new browser tab, open an additional instance of Clarity LIMS.
b. Navigate to the Consumables configuration screen.
c. Select the NaOH reagent kit and select New Lot.
d. Set the Status of Reagent Lot toggle switch to Active.
e. Enter the lot details.
f. Select Save.
g. Repeat this process to add and activate Resuspension Buffer and Tris-HCl reagent lots.
h. Return to your original working browser tab and refresh the page.

6. In the Reagent Lot Tracking section, select from the active lots displayed in each drop-down list.
7. The fields displayed on the Record Details screen are used to set up the run and generate the sample sheet. Some of these fields are autopopulated and some must be completed manually.

Table 116 Fields Displayed on Record Details Screen of Dilute and Denature (NovaSeq 6000 v3.8)

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.
BaseSpace Sequence Hub Configuration	Presets • Not Used • Run Monitoring Only • Run Monitoring and Storage
Workflow	Preset • GenerateFASTQ
Workflow Type	Presets • No Index • Single Index • Dual Index • Custom
Index Read 1	Presets • 0 • 6 • 8 • type a value between 0 and 20

Field	Value
Index Read 2	Presets • 0 • 6 • 8 • type a value between 0 and 20
Paired End	Presets • True • False
Read 1 Cycles	Presets • 251* • 151 • 101 • 51 • type a value between 1 and 251 *The value of 251 is only supported for SP flow cell type. For all other flow cell types, the maximum value is 151.
Read 2 Cycles	Presets • 251* • 151 • 101 • 51 • type a value between 0 and 251 *The value of 251 is only supported for SP flow cell type. For all other flow cell types, the maximum value is 151.
Use Custom Read 1 Primer	Select if applicable.
Use Custom Read 2 Primer	Select if applicable.
Use Custom Index Read 1 Primer	Select if applicable.
Reverse Complement Workflow	Select Yes (by default) for v1.5 reagents in NovaSeq Control Software v1.7 and onwards. Select No for v1 reagents.
Use Custom Recipe	Select if applicable.
Custom Recipe Path	If the Use Custom Recipe option is selected, enter the path to the custom recipe file to be used.
UMI - Read 1 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 1. Leave blank if not applicable.

Field	Value
UMI - Read 2 Length	Enter the length of the UMI in Read 2. Leave blank if not applicable.
UMI - Read 1 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.
UMI - Read 2 Start From Cycle	Enter the cycle number that Read 2 starts from. Leave blank if not applicable.
Output Folder	Enter network path for sequencing run folder (eg, `\\networkshare\run_data`)
Samplesheet Format	Presets • V1 • V2
Analysis Software Version	Enter the software version for BCL Convert Analysis for v2 sample sheet. Leave blank if not applicable.
Override Cycles	Enter the Override Cycles value for v2 sample sheet. Leave blank if not applicable.

8. On the Record Details screen, select Validate Run Setup and Generate Sample Sheet. This selection triggers the automation script, which performs the following actions:
   - Validates the parameters entered on the Record Details screen.
   - Generates the sample sheet and attaches it to the placeholder in the Files area of the Record Details screen.

     The v2 sample sheet generated does not include Cloud and Analysis sections. Contact Illumina Support for information on generating v2 sample sheets with those sections.

   - Select Next Steps.
9. On the Assign Next Steps screen, the Next Step for samples is prepopulated with Remove from workflow. The Routing Script automation expects this value, and requires the value to advance samples to the next step.

   Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.

10. Select Finish Step.
    After exiting the step, the following actions occur:
    - The Routing Script & Register Step Completed automation is triggered and samples are routed to AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8).
    - In Lab View, the pool of samples is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8) step.

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At this point in the workflow, the user interaction ends. Proceed to AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8).

Do not add samples to the Ice Bucket or start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8) step. The integration adds samples automatically.

Protocol 3: NovaSeq Xp (NovaSeq 6000 v3.8)

In this protocol, samples are pooled and added to lanes on the NovaSeq flow cell. The option selected in the Define Run Format (NovaSeq 6000 v3.8) step determines the flow cell type.

Step 1: Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.8)

1. In Lab View, locate the NovaSeq Xp (NovaSeq 6000 v3.8) protocol. The samples are queued for the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.8) step.
2. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Pooling screen, create and name a pool as follows.
   a. Create a pool by dragging samples into the Pool Creator.

      Create only one pool.

   b. Enter a name for the pool or accept the default name (Pool #1).
   c. Select Record Details.
5. On exiting the Pooling screen, the Validate Inputs Flowcell Type and Single Pool automation is triggered. The automation verifies the following items:
   - All samples in the pool are assigned the same Flowcell Type.
   - Only one pool was created.
6. On the Record Details screen in the Step Details area, complete the two required fields and one optional field, as needed:
   - Number of Lanes to Sequence—Used in volume calculations to make sure that volumes are sufficient for the number of times the pool is sequenced.
   - Minimum Per Sample Volume (µl)—Used to calculate how much of each sample is included in the pool. The field is prepopulated with the configured default value 5 µl, but can be edited.
   - % PhiX (0.25nM) Spike-In—Used to calculate the volume of PhiX v3 control to be included in the pool for the given percentage of spike-in. The field is optional and can be edited.

Assuming the default Minimum Per Sample Volume (µl) value of 5, for a given batch:

- If the smallest Per Sample Volume (µl) value is < 5, Clarity LIMS automatically assigns a value of 5 to the samples Adjusted Per Sample Volume (µl) field.
- Clarity LIMS then adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch. This adjustment is based on the ratio used to increase the lowest value to 5.

7. In the Sample Details table, select the pool icon to view details on the pool composition.
8. Select Calculate Volumes. This selection triggers the Calculate Volumes automation. This automation performs volume calculations based on the selected Flowcell Type, as follows.
   - Calculates Bulk Pool Volume (µl) value.
   - Calculates the Per Sample Volume (µl) value to be added to the pool.
   - Calculates PhiX Volume (µl) value when % PhiX (0.25nM) Spike-In has value.
   - Calculates the Total Sample Volume (µl) value.
   - If the Total Sample Volume is less than the Bulk Pool Volume, calculate the RSB Volume (µl) value.
   - Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
   - Generates the Calculation File (CSV) and attaches it to the step. This file contains volume information about the samples and RSB buffer to add to the pool. Select the file to download it and then open it in Excel.
9. Select Next Steps to trigger the Set Next Steps automation. This automation performs the following actions:
   - Copies the Flowcell Type values from the step inputs to the step outputs.
   - Sets the next step for samples to ADVANCE, advancing them to the Dilute, Denature & ExAmp (NovaSeq 6000 v3.8) step in the protocol.

   On the Assign Next Steps screen, the next step for samples is set to the Dilute, Denature & ExAmp (NovaSeq 6000 v3.8) step in the workflow.
10. Select Finish Step.
    At the end of this step, the pool of samples automatically advances to the Dilute, Denature & ExAmp (NovaSeq 6000 v3.8) step.

Step 2: Dilute, Denature & ExAmp (NovaSeq 6000 v3.8)

1. In Lab View, locate the NovaSeq Xp (NovaSeq 6000 v3.8) protocol. The pool of samples queued for the Dilute, Denature & ExAmp (NovaSeq 6000 v3.8) step displays.
2. Add the pool to the Ice Bucket and select View Ice Bucket.

3. [Optional] On the Ice Bucket screen, set the number of derivatives to create (placed into the flow cell lanes) and select Begin Work.
4. On entry to the Record Details screen, the Calculate Volumes automation is triggered.
   This automation sets the following values based on the selected Flowcell Type:
   - BP Aliquot Volume (µl)
   - NaOH Volume (µl)
   - Tris-HCl Volume (µl)
   - DPX1 Volume (µl)
   - DPX2 Volume (µl)
   - DPX3 Volume (µl)
   - Mastermix per Lane (µl)
   - The automation also populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
   - The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains information about the DPX Mastermix volume and the volume of Mastermix, NaOH, and Tris-HCl to add per working pool (see the next step).
5. On the Record Details screen, the Reagent Lot Tracking section tracks the DPX1, DPX2, DPX3, NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. These reagents must be added.
   To add and activate reagent lots:
   a. In a new browser tab, open an additional instance of Clarity LIMS.
   b. Select one of the reagent kits previously listed and select New Lot.
   c. Enter the lot details.
   d. Set the Status of Reagent Lot toggle switch to Active.
   e. Select Save.
   f. Repeat this process to add and activate the remaining reagent lots.
   g. Return to your original working browser tab and refresh the page.
6. On the Record Details screen:
   - In the Reagent Lot Tracking section, select from the active lots displayed in each drop-down list.
   - In the Step Details area, the DPX1, DPX2, and DPX3 reagent volume values are prepopulated. The script sets the values and they are not editable.
   - In the Sample Details table, select the pool icon to view details on the working pool composition.
     - The BP Aliquot, Mastermix per lane, NaOH, and Tris-HCl volume values for each working pool are populated. The script sets the values and they are not editable.

- The Flowcell Type and Loading Workflow Type columns are populated.
- The working pool number is appended to the bulk pool name, to identify which working pools are derived from the same bulk pool.

7. In the Files area, select the Calculation File (CSV) to open it and view details on the DPX Mastermix volume and the volume of Mastermix, NaOH, and Tris-HCl to add per working pool.
8. Select Next Steps. On the Assign Next Steps screen, the next step is already set to Load to Flowcell (NovaSeq 6000 v3.8).
9. Select Finish Step.

Step 3: Load to Flowcell (NovaSeq 6000 v3.8)

1. On the Ice Bucket screen:
   - In the Container Options panel, select the appropriate flow cell type from the Destination Container drop-down list.
   - Select Begin Work.
2. The Validate Inputs and Selected Container & Register Step Started automation checks the following:
   - The Flowcell Type field is set to a valid value (SP, S1, S2, or S4) and that each input has the same value for the field.
   - The container type selected matches the value in the Flowcell Type field.
   - The number of outputs matches the number of lanes on the selected flow cell type. If validation fails, an error message reports the number of working pools does not match the number of lanes available on the flow cell.
3. On the Placement screen:
   - Drag one or more pools from the left of the screen over into the Placed Samples area on the right.
   - Scan or type the barcode of the flow cell into the Flowcell field.
   - Select Record Details.

   After exiting the Placement screen, the Validate Flowcell Barcode automation makes sure that the container barcode conforms to the barcode mask for the selected Flowcell Type (as listed in the following table). This automation also copies the Flowcell Type and Loading Workflow Type field values from step inputs to outputs.

Flowcell Type	Barcode Mask
SP	[A-Z0-9]{5}DR[A-Z0-9]{2}
S1	[A-Z0-9]{5}DR[A-Z0-9]{2}

Flowcell Type	Barcode Mask
S2	[A-Z0-9]{5}DM[A-Z0-9]{2}
S4	[A-Z0-9]{5}DS[A-Z0-9]{2}

4. The fields displayed on the Record Details screen are used to set up the run and generate the sample sheet.
   Some of these fields are autopopulated and some must be entered manually. For details, refer to the following table:

   Table 117 Fields Displayed on Record Details Screen of Load to Flowcell (NovaSeq 6000 v3.8) Step

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.
BaseSpace Sequence Hub Configuration	Presets • Not Used • Run Monitoring Only • Run Monitoring and Storage
Workflow	Preset • GenerateFASTQ
Workflow Type	Presets • No Index • Single Index • Dual Index • Custom
Index Read 1	Presets • 0 • 6 • 8 • type a value between 0 and 20
Index Read 2	Presets • 0 • 6 • 8 • type a value between 0 and 20
Paired End	Presets • True • False

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Field	Value
Read 1 Cycles	Presets • 251* • 151 • 101 • 51 • type a value between 1 and 251 *The value of 251 is only supported for SP flow cell type. For all other flow cell types, the maximum value is 151.
Read 2 Cycles	Presets • 251* • 151 • 101 • 51 • type a value between 0 and 251 *The value of 251 is only supported for SP flow cell type. For all other flow cell types, the maximum value is 151.
Use Custom Read 1 Primer	Select if applicable.
Use Custom Read 2 Primer	Select if applicable.
Use Custom Index Read 1 Primer	Select if applicable.
Reverse Complement Workflow	Select Yes (by default) for v1.5 reagents in NovaSeq Control Software v1.7 and onwards. Select No for v1 reagents.
Use Custom Recipe	Select if applicable.
Custom Recipe Path	If you selected the Use Custom Recipe option, enter the path to the custom recipe file to be used.
UMI - Read 1 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 1. Leave blank if not applicable.
UMI - Read 2 Length	Enter the length of the UMI in Read 2. Leave blank if not applicable.
UMI - Read 1 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.
UMI - Read 2 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.

Field	Value
Output Folder	Enter network path for sequencing run folder (eg, `\\networkshare\run_data`)
Library Tube Barcode	Scan the library tube barcode.
Samplesheet Format	Presets • V1 • V2
Analysis Software Version	Enter the software version for BCL Convert Analysis for v2 sample sheet. Leave blank if not applicable.
Override Cycles	Enter the Override Cycles value for v2 sample sheet. Leave blank if not applicable.

5. Select Validate Run Setup and Generate Sample Sheet to trigger the automation script, which does the following:
   - Validates the parameters entered on the Record Details screen.
   - Generates the sample sheet and attaches it to the placeholder in the Files area on the Record Details screen.

     The v2 sample sheet generated does not include Cloud and Analysis sections. Contact Illumina Support for information on generating v2 sample sheets with these sections.
6. Select Next Steps.
   On the Assign Next Steps screen, the Next Step field for samples is prepopulated with Mark protocol as complete.
7. Select Finish Step.
   At this point in the workflow, the user interaction ends. The flow cell is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8) step.

Proceed to AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8).

Do not add samples to the Ice Bucket or start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8) step. The integration adds samples automatically.

Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8)

This protocol contains a single fully automated step - AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8).

The integration starts the step automatically and data from the run is parsed back into Clarity LIMS. User interaction is not required, but the various stages can be reviewed in Clarity LIMS under Review Run Data.

Automations

The NovaSeq Run (NovaSeq 6000 v3.8) step contains two automations:

- Set Next Steps—This automation is disabled (Set to Not Used). By default, the Sequencer API is used to set the next step for output samples.
  The Illumina Preset Protocols (IPP) include a Set Next Steps automation configured on the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8) step. You can use this automation to override the default next step behavior. For details, refer to *NovaSeq 6000 Integration v3.6.0 Configuration and User Interaction* on page 266for details.
- Update Lane Number—This automation is triggered automatically upon entry to the Record Details screen.
  Do not disable or modify this automation to make sure that the Lane Number displays properly.

Review Run Data

Read summary metrics are recorded for the library pool. After the run is complete, open the step and review these metrics on the Next Steps screen in the Step Data section and the Sample Details table.

Step Data Section

The following values populate in the master step fields:

- Run ID
- Run Status
- Output Folder
- Current Cycle
- Current Read
- Loading Workflow Type
- Flow Cell ID
- Flow Cell Side
- Flow Cell Mode
- Flow Cell Part Number
- Flow Cell Lot Number
- Flow Cell Expiration Date
- Instrument ID
- Instrument Type
- Instrument Control Software Version
- Firmware Version

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- RTA Version
- Sequencing Log

Sample Details Table

Summary metrics populate the global fields listed. Values are aggregated across all lanes. Some values (for example Yield PF (Gb) R1) are summed, while others are averaged.

- Yield PF (Gb) R1
- Yield PF (Gb) R2
- Reads PF (M) R1
- Reads PF (M) R2
- Cluster Density (K/mm^2) R1
- Cluster Density (K/mm^2) R2
- %PF R1
- %PF R2
- % Bases >=Q30 R1
- % Bases >=Q30 R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- % Aligned R1
- % Aligned R2
- % Error Rate R1
- % Error Rate R2

How the Integration Works

The following steps summarize how the Sequencer API integration works. For details on sample sheet generation and the sequence of events involved in the NovaSeq run, refer to *NovaSeq 6000 Integration v3.6.0 Configuration and User Interaction* on page 266.

1. When setting up a run on the NovaSeq 6000 instrument, NVCS sends a request for the run recipe.
    a. The Sequencer API validates that samples and containers are correctly queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8) step.

b. Clarity LIMS sends a JSON response to NVCS. The response includes the run recipe information and a link to download the sample sheet (used with analysis software such as bcl2fastq2).

2. When the run starts, NVCS sends a RunStarted run status request. The Sequencer API then does the following:
   a. Validates reagent kit information in the RunStarted request:
      - Verifies that the reagent kit exists. If the reagent kit does not exist, the Sequencer API creates and enables it on the master step.
      - Verifies that the reagent kit is activated. If the reagent kit is not active, the Sequencer API activates it.
   b. Initiates the step for the queued samples. The step produces one output file placeholder per lane of the flow cell in use, based on the flow cell type.
   c. Records all relevant information from the RunStarted request on the step (such as reagent lots and step fields).
3. At the end of the run, NVCS sends one of the following run status requests:
   - RunCompletedSuccessfully
   - RunEndedByUSer
   - RunErroredOut

   The Sequencer API updates the step with any new information, which includes only the status and cycle/read information.
4. After primary analysis completes, NVCS sends a request containing the parsed run metrics. NVCS sends a request only when the run completed successfully, but the API does not make this assumption and accepts the request regardless of status. The Sequencer API then does the following actions:
   a. Records the metrics into the fields on file placeholder outputs in the LIMS.
   b. Completes the step in Clarity LIMS when the status is RunCompletedSuccessfully. For all other status options, the step remains in progress. This action is the default autocomplete step behavior. For details, refer to *NovaSeq 6000 Integration v3.6.0 Configuration and User Interaction* on page 266.

   > *NovaSeq 6000 Integration v3.6.0 Configuration and User Interaction* on page 266 provides detailed information on how the following components work with the Sequencer API integration:
   >
   > - Sample sheet generation and contents
   > - NovaSeq 6000 run
   > - User authentication and login
   > - Run recipe contents

Troubleshooting

If an automation trigger does not appear to run its corresponding scripts, refer to the following sections in the Clarity LIMS API documentation:

- *Troubleshooting Automated Informatics*
- *Troubleshooting Automation in the API*

If an error occurs that does not provide direction on how to proceed, complete the following steps:

1. Confirm the version of the installed Illumina NovaSeq 6000 Integration Package by running the following command on the Clarity LIMS server command line:

```
rpm -qa | grep -i novaseq
```

2. If the error is related to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8) step, review the log file information. For details, refer to *Logging* on page 350.

If the automated run step starts but does not complete, proceed as follows.

1. Use one of the following methods to open the in progress step in Clarity LIMS:
   - Log in to the default user account.
   - In Lab View, find the step in the Recent Activities pane.
   - Search for the step in Clarity LIMS, using either the Library Tube, or Flowcell barcode as the search term. The steps depend on whether the search is a NovaSeq Standard or NovaSeq Xp run.
2. On the Record Details screen, the Sequencing Log multiline text field contains logging information. If you are unable to reach the Record Details screen, or if the Sequencing Log field does not contain enough information to resolve the issue, review the `sequencer-api.log` file. For details, refer to *Logging* on page 350.
3. Contact Illumina Support. Provide the relevant information from the troubleshooting steps already performed.

Logging

In addition to updating the Sequencing Log multiline text field on the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8) step, the Sequencer API writes a detailed log file to the following location:

```
/opt/gls/clarity/tomcat/current/logs/sequencer-api.log
```

Log messages include the Library Tube ID and Flowcell ID whenever the messages are related to a sequencing run-related request. In the LIMS workflows, these IDs are recorded as container names.

Some log messages are not directly related to a sequencing run request, such as downloading the sample sheet. In this case, the file LIMS ID and file name are included in log messages. The sample sheet downloaded by the sequencing system has a name that includes the sequencing container ID (Library Tube for Standard or Flowcell for Xp). The relevant container name search finds most the log

messages for this request. However, if all messages for downloading a file are found, the LIMS ID of the file must be known. The LIMS ID is found in the Clarity LIMS API and, for sample sheets used in a run, in the recipe response (also included in the log file).

Example lines from the log file:

```
2021-06-19 21:32:19.705 INFO --- [] SequencerAPIApplication : Started
SequencerAPIApplication in 7.026 seconds (JVM running for 15.964)
...
2021-06-19 21:34:44.564 INFO --- [Library Tube Id=NV1234567-LIB, Flow Cell
Id=RM123DSXX] LimsApiLookupUtil : Searching for containers with name 'NV1234567-
LIB' or 'RM123DSXX'
....
2021-06-19 21:35:27.158 INFO --- [] FileService : Successfully retrieved file
'NV1234567-LIB.csv' with LIMS ID 40-51 through the API.
```

NovaSeq 6000 Integration v3.6.0 Installation

The Illumina NovaSeq 6000 Integration v3.6.0 supports the integration between Clarity LIMS (v6.2 and later) and the NovaSeq 6000 instrument. This integration also includes updates to the technology stack and third party libraries and utilities. The key changes from v3.5.1 are as follows.

- Updated the preconfigured NovaSeq 6000 workflow to include Clarity LIMS Product Analytics (CLPA) automation scripts by default.
- Compatibility with Oracle Linux. For compatibility, refer to *NovaSeq 6000 Integration v3.6 Release Notes* on page 368.

Clarity LIMS is automatically stopped during installation of the RPM and will need to be restarted.

The file-based and API-based integrations cannot run at the same time. When the API-based integration has finished installing, shut down Clarity LIMS and uninstall the file-based integration.

This document provides instructions for installing NovaSeq 6000 Integration v3.6.0. It also describes the components that are installed in the default configuration.

- If you are installing the NovaSeq 6000 integration for the first time, follow the instructions provided in this document.
- If NovaSeq 6000 Integration v3.5.1 is already installed, with only minor changes to the default configuration, follow the instructions provided in this document and then duplicate the configuration changes from the v3.5.1 workflow.
- If there have been significant changes to the default configuration, follow the instructions provided in *NovaSeq 6000 Integration v3.6.0 Manual Upgrade* on page 360.

Integration Overview

Illumina NovaSeq 6000 Integration v3.6.0 includes the following features and functionality.

- Preconfigured NovaSeq 6000 v3.8 workflow that maps to lab protocols and instrument runs.
- The following preconfigured protocols:
  - Run Format (NovaSeq 6000 v3.8)
  - NovaSeq Standard (NovaSeq 6000 v3.8) (supports the loading of pooled libraries into a library tube)
  - NovaSeq Xp (NovaSeq 6000 v3.8) (supports individual lane loading on the NovaSeq 6000)
  - AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8)
- Automated generation of the v1 or v2 sample sheet. This file is automatically uploaded to the sequencing instrument through the Sequencer API and is used to set up the run.
- Automated validation of run setup information. This information is uploaded to the NovaSeq Control Software (NVCS) through the Sequencer API. It is used to create the run recipe and initiate the run.
- Automated tracking of the following NovaSeq sequencing runs and parsing of run statistics into Clarity LIMS:
  - Progress and metrics of sequencing run
  - Per-instrument sequencing runs (tracked as part of the Run ID field value)
  - Sequencing run parameters
  - Sequencing run data directory location
  - Real-Time Analysis v3 (RTA3) version and other run-specific information
- [Optional] Preconfigured Library Prep Validation v2.3.1 workflow used for validation purposes. This workflow contains a single-step protocol that models the library prep required to produce normalized libraries that are ready for the NovaSeq 6000 v3.8 workflow. For more information, refer to *NovaSeq 6000 Integration v3.6.0 Validation and Troubleshooting* on page 331.

Supporting Documentation

- For details on installed protocols and steps, automations, generated and captured files, and rules and constraints, refer to *NovaSeq 6000 Integration v3.6.0 Configuration and User Interaction* on page 266.
- For information on validating and troubleshooting the integration, refer to *NovaSeq 6000 Integration v3.6.0 Validation and Troubleshooting* on page 331.
- For information on the NovaSeq 6000 instrument system, refer to the *NovaSeq 6000 System Support Resources*.

Prerequisites

NovaSeq 6000 Integration v3.6.0 has the following prerequisites:

Prerequisite 1: Secret Utility Configuration

If Clarity LIMS Secret Utility is not installed or configured, use the following information to configure Secret Utility:

Installation on a hosted system with Clarity LIMS (v6.2 or later)

The Clarity LIMS installation tooling configures this installation. No additional configuration is necessary.

Installation on an on-premise Clarity LIMS instance (v6.2 or later)

The installation requires the following passwords:

- Tenant DB Password
- Clarity DB Password
- FTP password
- LDAP manager password (if LDAP is enabled)
- LabLink DB password
- Reporting DB password (if installed)
- RabbitMQ password

For more information on Secret Utility configuration, refer to the *Clarity LIMS Core Functionality* documentation.

Prerequisite 2: Illumina Preset Protocols (IPP) v2.6

The integration package depends on the configuration provided in Illumina Protocols (IPP) v2.6.

The installation procedure provided in this document includes steps that install the following IPP configurations on the server used for the NovaSeq 6000 integration installation:

- NovaSeq configuration
- Library Prep Validation configuration (optional, but recommended)

For details on IPP v2.6 installation and configuration, refer to the *Illumina Preset Protocols* documentation.

Prerequisite 3: Proxy Update (Illumina Cloud Hosted Installation Only)

The Illumina cloud hosted installation configures an additional include directive for `/etc/httpd/conf.d/clarity.conf` in the proxy configuration. Additional proxy configuration files for the Sequencer API service are expected to be placed in the `/etc/httpd/conf.d/clarity` folder.

Sequencer API RPM Installation Components

NovaSeq Integration v3.6.0 is distributed as the BaseSpaceLIMS-sequencer-api RPM package. This package must be installed on the Clarity LIMS server.

The BaseSpaceLIMS-sequencer-api RPM installs the following files and scripts:

- Sequencer API WAR file
- `application.yml` configuration file
- `configure_sequencer_api_proxy.sh` configuration script
- `configure_sequencer_api_env.sh` configuration script
- `configure_sequencer_api_application.sh` configuration script

Sequencer API WAR

The RPM installs the Sequencer API WAR file into the same Tomcat container as Clarity LIMS. The WAR file is at `/opt/gls/clarity/tomcat/current/webapps/Illumina#Sequencer#v2##2.6.0.0.war`. The version number can differ.

The WAR file is deployed automatically when Clarity LIMS launches. Because the RPM installs a new WAR file, Clarity LIMS is automatically stopped during installation of the RPM. Restart Clarity LIMS before installing the NovaSeq and Library Prep Validation workflow configuration from the IPP.

Configuration File

The `application.yml` configuration file is installed at `/opt/gls/clarity/extensions/sequencer-api/application.yml`. As part of the installation process, the file is configured with one of the configuration scripts.

For a complete list of all properties included in the `application.yml` file, refer to *NovaSeq 6000 Integration v3.6.0 Configuration and User Interaction* on page 266

Configuration Scripts

The scripts in the following table are installed at the `/opt/gls/clarity/config/` folder. During installation, the RPM runs these scripts as they are needed.

Script Name	User	Description
configure_sequencer_api_proxy.sh	root	Adds configuration to the proxy to allow communication with the Sequencer API after Tomcat has been started. Run this script in the following scenarios: • When the Sequencer API is first installed. • When reconfiguring the Clarity LIMS proxy. This process happens until a version of Clarity LIMS that includes the necessary configuration files from `/etc/httpd/clarity/*.conf` is available. Not required for Illumina cloud hosted installations.
configure_sequencer_api_env.sh	root	Adds Secret Management setting into the Clarity LIMS Tomcat configuration. Run this script when the Sequencer API is first installed on a Secret Util-enabled Clarity LIMS.

Script Name	User	Description
configure_sequencer_api_application.sh	glsjboss	• Configures the installed `application.yml` file. • Sets the Clarity LIMS URL and the key to use when signing access tokens. Run this script when the Sequencer API is first installed. If you run the script multiple times, the script reconfigures each setting to its default. The only exception is the signing key. For this setting, you are asked if you want a reset. If the reset is done, all previously issued tokens are no longer valid.

Installation Steps

If running the sequencing service and Automation Worker on the same instance, the instance must also be running a compatible version of Oracle Linux. For compatibility, refer to *NovaSeq 6000 Integration v3.6 Release Notes* on page 368.

Install the IPP Package

1. On the server being used to install the integration, log in as the root user.
2. Use the following yum command to install the IPP RPM:

```
yum install BaseSpaceLIMS-Illumina-Preset-Protocols --enablerepo=<< repo name info from support >>
```

   The `--enablerepo` command-line argument must be included to enable the repo. The Illumina Support team can provide you with the repo file with the appropriate naming convention.
3. Enter `y` to confirm that you want to proceed with the RPM and package installation.
4. Make sure that NGS Extension Package v5.23 is installed.
   If this package is not installed, install it or upgrade the existing package.

For information on IPP installer commands, parameters, and options, refer to the *Illumina Preset Protocols* documentation.

Install Sequencer API

Install the Sequencer API as follows.

1. On the Clarity LIMS server, log in as the root user.
2. Use the following yum command to install the RPM:

```
yum install BaseSpaceLIMS-sequencer-api --enablerepo=< repo name info from
support >
```

   The `--enablerepo` command-line argument must be included to enable the repo. The Illumina Support team can provide you with the repo file with the appropriate naming convention.

3. Enter `y` to confirm that you want to proceed with the RPM installation.
4. If necessary, install the Secret Utility Package.
   For more information on this package, refer to *Prerequisite 1: Secret Utility Configuration* on page 353.
5. As the glsjboss user, run configure_sequencer_api_application.sh from the following location:

```
bash /opt/gls/clarity/config/configure_sequencer_api_application.sh
```

6. Enter values for the following questions:
   You can also accept the default values in the `application.yml` configuration file by pressing Enter. For more information about the `application.yml` properties, refer to *NovaSeq 6000 Integration v3.6.0 Configuration and User Interaction* on page 266.
   - Would you like to use the apiuser user for communication with Clarity? [Y]
   - Enter required value for Token Expiry (hours) [88]
7. Update the Clarity LIMS proxy for the Sequencer API as follows.
   - For an on-premise system, run the following command:

```
bash /opt/gls/clarity/config/configure_sequencer_api_proxy.sh
```

   - For an Illumina cloud hosted system, make sure that the proxy has been updated as follows. Place the `sequencer-api.include` file in `/etc/httpd/conf.d/clarity`.

```
<Location "/Illumina/Sequencer/v2">
ProxyPass ajp://localhost:9009/Illumina/Sequencer/v2
ProxyPassReverse ajp://localhost:9009/Illumina/Sequencer/v2
</Location>
```

8. Update the Clarity LIMS Tomcat configuration as follows.
   a. Make sure that the Secret Util is installed and configured. For more information, refer to *Prerequisite 1: Secret Utility Configuration* on page 353.
   b. Run the following command to add the Secret Utility configuration to Clarity LIMS:

```
bash /opt/gls/clarity/config/configure_sequencer_api_env.sh
```


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> This script modifies `/opt/gls/clarity/tomcat/current/bin/setenv.sh` for Clarity LIMS Tomcat to load the Secret Utility environment variable file (`/etc/profile.d/claritysecret.sh`) during startup.

c. Use the following command to Start Clarity LIMS:

```
/opt/gls/clarity/bin/run_clarity.sh start
```

Install NovaSeq and Library Prep Validation Workflow Configuration

Install the NovaSeq and Library Prep workflow configuration from IPP as follows.

1. As the glsjboss user, run the following command to install the NovaSeq 6000 v3.8 workflow configuration:

```
bash /opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o install
Illumina_Instruments.novaseq-v3.8
```

> The installer validates the import of the workflow and provides the Warning/Error details in STDOUT, which allows you to either proceed with the import or cancel it.

2. [Optional] Run the following command to install the Library Prep Validation v2.3.1 workflow:

```
/opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o install
Illumina_Instruments.LibraryPrepValidation-v2.3
```

This step is optional, but recommended. The Library Prep Validation v2.3.1 workflow allows you to validate the NovaSeq 6000 v3.8 workflow.

Check Sequencer API

Make sure that the Sequencer API is working as follows.

1. Visit `https://<< Clarity LIMS URL >>/Illumina/Sequencer/v2/sequencing-run/login`. This page returns the following URL string:

```
https://<< Clarity LIMS URL >>/Illumina/Sequencer/v2/oauth/authorize?client_
id=illumina_sequencer&amp;redirect_uri=../sequencer_login&amp;response_
type=token
```

2. Copy and paste the URL string into the browser. The Clarity LIMS Sign In page opens.
3. Enter sign in credentials.
4. Select Sign In. A Successfully Authenticated message displays.

[Optional] Configure application.yml Properties

The installer script configures the `application.yml` configuration properties by prompting for values. You can also configure these properties by editing the `application.yml` file, and then restarting Clarity LIMS. Configuring these properties is optional.

For more information, refer to *NovaSeq 6000 Integration v3.6.0 Configuration and User Interaction* on page 266.

Workflows, Protocols, and Steps Installed

NovaSeq 6000 Integration v3.6.0 includes the workflows, protocols, and steps listed in the following table.

Package	Workflow	Protocols	Steps
IPP v2.6	NovaSeq 6000 v3.8	Run Format (NovaSeq 6000 v3.8)	1. Define Run Format (NovaSeq 6000 v3.8)
		NovaSeq Standard (NovaSeq 6000 v3.8)	1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.8) 2. Dilute and Denature (NovaSeq 6000 v3.8)
		NovaSeq Xp (NovaSeq 6000 v3.8)	1. Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.8) 2. Dilute, Denature & ExAmp (NovaSeq 6000 v3.8) 3. Load to Flowcell (NovaSeq 6000 v3.8)
		AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8)	1. AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8)
	Library Prep Validation v2.3.1	Library Prep Validation v2.3.1	1. Library Prep Validation v2.3.1

For detailed descriptions of the steps and automations included in each protocol, and details on other components in the configuration, refer to *NovaSeq 6000 Integration v3.6.0 Configuration and User Interaction* on page 266.

For instructions on using the Library Prep Validation v2.3.1 protocol, refer to *NovaSeq 6000 Integration v3.6.0 Validation and Troubleshooting* on page 331.

The configuration provided in this integration has been established to support NovaSeq 6000 lab processes. Any configuration changes to protocols or workflows, including renaming protocols, steps, and fields, could break process.

Instrument Software

The NovaSeq instrument software includes the following components:

- NovaSeq Control Software (NVCS)—Contains the user interface for setting up the sequencing run. Responsible for controlling the instrument and acquiring the images.
- Real-Time Analysis v3 (RTA3)—Takes the images generated by the first module, processes, and analyzes them. Makes sure that data files are created and copied to the final destination folder.
- Sequencing Analysis Viewer (SAV)—Displays the important quality metrics generated by the RTA3 software.
- NovaSeq recipes—Provides system operation instructions for use with NovaSeq 6000 reagent kits for SP, S1, S2, and S4 flow cells.
- Universal Copy Service—Copies output files to destinations such as final destination folder and/or BaseSpace Sequence Hub (when the instrument is configured for use with BaseSpace Sequence Hub).

Instrument Integration Configuration

For the NovaSeq API integration to work, the NovaSeq instrument must be able to communicate with Clarity LIMS through the API. Complete the following steps to configure the NVCS and confirm that you can access Clarity LIMS from the instrument.

1. Launch NVCS and wait for the initialization process to complete.
2. On the NVCS Settings page, do the following:
   a. Under Mode Selection, select Server-Based.
   b. In the adjacent field, enter the Clarity LIMS URL (eg, `https://customerserver.claritylims.com`).
   c. [Optional] In the Output Folder field, enter the default output folder location. You can also specify the output folder location when setting up a run in NVCS.
3. Select Save to complete the configuration.
4. On the NVCS home page, you can now select Sequence to log into Clarity LIMS and start a run.

NovaSeq 6000 Integration v3.6.0 Manual Upgrade

NovaSeq Integration Package v3.6.0 contains modifications to the existing automations from NovaSeq Integration Package v3.5.1. The NovaSeq Sequencing v3.8 workflow and Illumina Preset Protocols (IPP) v2.6 are required for the integration package to work.

If you do not want to upgrade the existing NovaSeq Sequencing workflow to the latest version through IPP, you can update the workflow configuration manually by completing the following steps.

The key change between NovaSeq 6000 v3.7 workflow and NovaSeq 6000 v3.8 workflow that comes with NovaSeq 6000 Integration v3.6.0 is the addition of Clarity LIMS Product Analytics (CLPA) enabled automation scripts. If CLPA is not required, it is not necessary to perform this manual upgrade.

Add New Automations

1. From Configuration, select the Automation tab.
2. Add the automations in the following table:

Table 118 Automations

Automation Name	Command Line	Master Steps
Register NovaSeq RunCompleted & Register Step Completed	`bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/novaseq6000/novaseq_seqrun_completed.groovy' && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/common/step_completed.groovy'"`	AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7)

Automation Name	Command Line	Master Steps
Register Pools & Register Step Completed	`bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/pooling.groovy' && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_completed.groovy'"`	Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.7) Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.7)
Register Step Completed	`bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_completed.groovy'"`	Dilute, Denature & ExAmp (NovaSeq 6000 v3.7) Load to Flowcell (NovaSeq 6000 v3.7)
Register Step Started[1]	`bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_started.groovy'"`	Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.7) Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.7)

Automation Name	Command Line	Master Steps
Register Step Started[2]	`bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/common/step_started.groovy'"`	Define Run Format (NovaSeq 6000 v3.7)

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Automation Name	Command Line	Master Steps
Register Step Started & Register NovaSeq Run & Register NovaSeq Run Association & Register NovaSeq RunStarted	`bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/common/step_started.groovy' && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/novaseq6000/register_novaseq_run.groovy' && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/novaseq6000/novaseq_associate_seqrun.groovy' && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/novaseq6000/novaseq_seqrun_started.groovy'"`	AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7)

[1] This automation has -l {compoundOutputFileLuid0} in the log file output index.
[2] This automation has -l {compoundOutputFileLuid1} in the log file output index.

Modify Routing Script Automation on Define Run Format (NovaSeq 6000 v3.7) Step

1. From Configuration, select the Automation tab.

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2. Under the Step Automation tab, select the Routing Script automation that is enabled on the Define Run Format (NovaSeq 6000 v3.7) master step.
3. In the automation name field, change the name to Routing Script & Register Step Completed.
4. Update the existing command line as follows.
   Changes are highlighted in red.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -u {username} -p {password} -i {stepURI:v2} -l {compoundOutputFileLuid0} script:changeWorkflow \
\
--FIELD_NAME 'Loading Workflow Type' \
--FIELD_VALUE 'NovaSeq Standard' \
--WORKFLOW 'NovaSeq 6000 v3.7' \
--STEP 'Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.7)' \
--INPUTS_OR_OUTPUTS 'INPUTS' \
\
--FIELD_NAME 'Loading Workflow Type' \
--FIELD_VALUE 'NovaSeq Xp' \
--WORKFLOW 'NovaSeq 6000 v3.7' \
--STEP 'Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.7)' \
--INPUTS_OR_OUTPUTS 'INPUTS' \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/common/step_completed.groovy'"
```

5. Select Save.

Modify Routing Script Automation on Dilute and Denature (NovaSeq 6000 v3.7) Step

1. From Configuration, select the Automation tab.
2. Under the Step Automation tab, select the Routing Script automation that is enabled on the Dilute and Denature (NovaSeq 6000 v3.7) master step.
3. In the automation name field, change the name to Routing Script & Register Step Completed.
4. Update the existing command line as follows.
   Changes are highlighted in red.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -u {username} -p {password} -i {stepURI:v2} -l {compoundOutputFileLuid0} script:changeWorkflow \
\
```

```
--FIELD_NAME 'Loading Workflow Type' \
--FIELD_VALUE 'NovaSeq Standard' \
--WORKFLOW 'NovaSeq 6000 v3.7' \
--STEP 'Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.7)' \
--INPUTS_OR_OUTPUTS 'INPUTS' \
\
--FIELD_NAME 'Loading Workflow Type' \
--FIELD_VALUE 'NovaSeq Xp' \
--WORKFLOW 'NovaSeq 6000 v3.7' \
--STEP 'Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.7)' \
--INPUTS_OR_OUTPUTS 'INPUTS' \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/common/step_completed.groovy'"
```

5. Select Save.

Modify Validate Inputs and Selected Container Automation on Load to Flowcell (NovaSeq 6000 v3.7) Step

1. From Configuration, select the Automation tab.
2. Under the Step Automation tab, select the Validate Inputs and Selected Container automation that is enabled on the Load to Flowcell (NovaSeq 6000 v3.7) master step.
3. In the automation name field, change the name to Validate Inputs and Selected Container & Register Step Started.
4. Update the existing command line as follows.
   Changes are highlighted in red.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar script:validate_flowcell_for_input_pools -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -validateSelectedContainer true && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_started.groovy'"
```

5. Select Save.

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Modify Validate Inputs Flowcell Type Automation on Dilute, Denature & ExAmp (NovaSeq 6000 v3.7) Step

1. From Configuration, select the Automation tab.
2. Under the Step Automation tab, select the Validate Inputs Flowcell Type automation that is enabled on the Dilute, Denature & ExAmp (NovaSeq 6000 v3.7) master step.
3. In the automation name field, change the name to Validate Inputs Flowcell Type & Register Step Started.
4. Update the existing command line as follows.
   Changes are highlighted in red.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar script:validate_flowcell_for_input_pools -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_started.groovy'"
```

5. Select Save.

Modify Validate Single Input Automation on Dilute and Denature (NovaSeq 6000 v3.7) Step

1. From Configuration, select the Automation tab.
2. Under the Step Automation tab, select the Validate Single Input automation that is enabled on the Dilute and Denature (NovaSeq 6000 v3.7) master step.
3. In the automation name field, change the name to Validate Single Input & Register Step Started.
4. Update the existing command line as follows.
   Changes are highlighted in red.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:validateSampleCount -min 1 -max 1 && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_started.groovy'"
```

5. Select Save.

Set Trigger Location and Style for New Automations

1. From Configuration, select the Lab Work tab.

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2. Change the trigger locations and styles for the automations in the following table:

Table 119 Automation Settings

Step Name	Automation Name	Trigger Location	Trigger Style
Define Run Format (NovaSeq 6000 v3.7)	Register Step Started	Step	Automatic upon entry
Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.7)	Register Step Started	Step	Automatic upon entry
	Register Pools & Register Step Completed	Step	Automatic upon exit
Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.7)	Register Step Started	Step	Automatic upon entry
	Register Pools & Register Step Completed	Step	Automatic upon exit
Dilute, Denature & ExAmp (NovaSeq 6000 v3.7)	Register Step Completed	Step	Automatic upon exit
Load to Flowcell (NovaSeq 6000 v3.7)	Register Step Completed	Step	Automatic upon exit
AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7)	Register Step Started & Register NovaSeq Run & Register NovaSeq Run Association & Register NovaSeq RunStarted	Step	Automatic upon entry
	Register NovaSeq RunCompleted & Register Step Completed	Step	Automatic upon exit

NovaSeq 6000 Integration v3.6 Release Notes

These release notes describe the key changes to software components for the Clarity LIMS NovaSeq 6000 Integration Package v3.6.

Compatibility

This version of the integration package is compatible with the following software:

- Clarity LIMS v6.2 and later
- NGS Extensions Package v5.23 and later
- Illumina Preset Protocols (IPP) v2.6.0 and later
- NovaSeq Control Software (NVCS) v1.8.0

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- Illumina bcl2fastq v2.20
- Oracle Linux (64-bit) v8.8

New Features

- Updates Groovy to v3.0.7.
- Replaces the non-CLPA enabled NovaSeq 6000 v3 workflow with the CLPA enabled workflow.

Defects Fixed

During configuration, you can now validate the existence of the input user instead of resetting the user and password.

Known Issues

None

NovaSeq 6000 Integration v3.5.1 Configuration and User Interaction

The Illumina NovaSeq 6000 Integration Package v3.5.1 supports the integration of Clarity LIMS to Illumina NovaSeq 6000 instruments. This documentation describes the integration and includes the following information:

- Preconfigured workflows, protocols, steps, and automations
- Installed components
- Configuration requirements, rules, and constraints

For instructions on validating and troubleshooting the Illumina NovaSeq 6000 Integration, refer to *NovaSeq 6000 Integration v3.5.1 Validation and Troubleshooting* on page 429.

> The configuration provided in this integration has been established to support NovaSeq 6000 lab processes. Any configuration changes to protocols or workflows - including renaming protocols, steps, and fields - could break process.

Overview

The integration includes the following features:

- Preconfigured NovaSeq 6000 v3.7 workflow that maps to lab protocols and instrument runs.
- The following preconfigured protocols:
  - Run Format (NovaSeq 6000 v3.7)
  - NovaSeq Standard (NovaSeq 6000 v3.7) (supports the loading of pooled libraries into a library tube)
  - NovaSeq Xp (NovaSeq 6000 v3.7) (supports individual lane loading on the NovaSeq)

  - AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7)
- Automated generation of v1 sample sheet for use with bcl2fastq2 v2.20 analysis software or v2 sample sheet. This file is automatically uploaded to the instrument through the API.
- Automatic validation of run setup information. This information is used to create the run recipe and initiate the run.
- Automated tracking of the NovaSeq sequencing run and parsing of run statistics into Clarity LIMS, including the following features:
  - Progress and metrics of sequencing run
  - Per-instrument sequencing runs (tracked as part of the Run ID field value)
  - Sequencing run parameters
  - Sequencing run data directory location
  - Real-Time Analysis v3 (RTA3) version and other run-specific information
- [Optional] Preconfigured Library Prep Validation v2.3.0 workflow used for validation purposes only. The workflow contains a single-step protocol that models the library prep required to produce normalized libraries that are ready for the NovaSeq 6000 v3.7 workflow. For more information, refer to *NovaSeq 6000 Integration v3.5.1 Validation and Troubleshooting* on page 429. The Library Prep Validation v2.3.0 workflow is available in IPP v2.5 Package.

Prerequisites and Assumptions

It is assumed that samples enter the NovaSeq 6000 v3.7 workflow as normalized libraries. It is assumed that the following steps have completed before samples are assigned to the workflow:

- Samples have been accessioned into the Clarity LIMS.
- Samples have been run through QC and library prep.
- Samples have been normalized, and the value is captured in a field called Normalized Molarity (nM).

For more information on sample accessioning, refer to *Sample Accessioning* and *Upload and Modify Samples* in the Getting Started section of the Clarity LIMS Core Functionality documentation.

You can assign samples to workflows automatically, using a routing script, or manually—from the Projects & Samples dashboard. Refer to *Assign and Process Samples* in the Clarity LIMS Core Functionality documentation.

Workflows, Protocols, and Steps

The Illumina NovaSeq 6000 Integration Package v3.5.1 includes the following workflows:

- NovaSeq 6000 v3.7
- Library Prep Validation v2.3.0 (optional, but recommended for validation purposes)

The following table describes the protocols and steps included in these workflows.

Table 120 NovaSeq 6000 Integration Package v3.5.1 Protocols and Steps

Protocol Name	Protocol Purpose	Steps Included in Protocol
	Library Prep Validation v2.3.0 Workflow	
Library Prep Validation v2.3.0	Included for validation purposes only, this protocol models the library prep steps required to advance samples to the Run Format (NovaSeq 6000 v3.7) protocol. The protocol contains a single step - Library Prep Validation v2.3.0. After this step, a routing script sends the samples to the first step of the NovaSeq 6000 v3.7 workflow - Define Run Format (NovaSeq 6000 v3.7).	1. Library Prep Validation v2.3.0
	NovaSeq 6000 v3.7 Workflow	
Run Format (NovaSeq 6000 v3.7)	Allows for the assignment of per sample values for: • Loading Workflow Type: Select either NovaSeq Standard or NovaSeq Xp. • Normalized Molarity: Enter a value for each sample. • Flowcell Type: Select from options SP, S1, S2, or S4. • Final Loading Concentration (pM): Select from options 225 (PCR-free workflows) or 400 (Nano workflows), or enter a different value. Compares the Normalized Molarity value of each sample with the Minimum Molarity value. Routing script sends samples to the NovaSeq Standard or NovaSeq Xp protocol, according to the selected Loading Workflow Type. Samples with Normalized Molarity less than Minimum Molarity are removed from the workflow.	1. Define Run Format (NovaSeq 6000 v3.7)

Protocol Name	Protocol Purpose	Steps Included in Protocol
NovaSeq Standard (NovaSeq 6000 v3.7)	Samples are pooled and added to the library tube in preparation for the NovaSeq run. The run setup information is validated and a sample sheet is generated. Routing script sends the library tube to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7) protocol.	1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.7) 2. Dilute and Denature (NovaSeq 6000 v3.7)
NovaSeq Xp (NovaSeq 6000 v3.7)	Samples are pooled and added to lanes on the NovaSeq flow cell. The option selected in the Define Run Format (NovaSeq 6000 v3.7) step determines the flow cell type. The run setup information is validated and a sample sheet is generated. Routing script sends flow cell to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7) protocol.	1. Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.7) 2. Dilute, Denature, and ExAmp (NovaSeq 6000 v3.7) 3. Load to Flowcell (NovaSeq 6000 v3.7)
AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7)	All samples complete the workflow by going through this protocol. This protocol contains one fully automated step. Do not add samples to the Ice Bucket or start the step. The integration starts the step automatically.	1. AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7)

Validation Workflow

The Library Prep Validation v2.3.0 workflow allows for validation of the system after installation is complete. For details, refer to *NovaSeq 6000 Integration v3.5.1 Validation and Troubleshooting* on page 429.

Step Automations

The following table lists the step automations included in the NovaSeq 6000 v3.7 workflow. Automation details are provided in the documentation for each step.

For details on the validation workflow, refer to *NovaSeq 6000 Integration v3.5.1 Validation and Troubleshooting* on page 429.

Table 121 NovaSeq 6000 v3.7 Workflow Step Automations

Protocol	Step	Automation
1. Run Format (NovaSeq 6000 v3.7)	1. Define Run Format (NovaSeq 6000 v3.7)	Routing Script Set Next Steps
2. NovaSeq Standard (NovaSeq 6000 v3.7)	1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.7)	Calculate Volumes Set Next Step Validate Inputs Flowcell Type and Single Pool Validate Unique Indexes (Not used. The core Clarity LIMS configuration for pooling handles this functionality.)
	2. Dilute and Denature (NovaSeq 6000 v3.7)	Routing Script Check Library Tube Barcode Validate Run Setup and Generate Sample Sheet Validate Single Input
3. NovaSeq Xp (NovaSeq 6000 v3.7)	1. Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.7)	Calculate Volumes Set Next Step Validate Inputs Flowcell Type and Single Pool Validate Unique Indexes (Not used. The core Clarity LIMS configuration for pooling handles this functionality.)
	2. Dilute, Denature, and ExAmp (NovaSeq 6000 v3.7)	Calculate Volumes Set Next Step Validate Inputs Flowcell Type
	3. Load to Flowcell (NovaSeq 6000 v3.7)	Validate Flowcell Barcode Validate Inputs and Selected Container Validate Run Setup and Generate Sample Sheet

Protocol	Step	Automation
4. AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7)	1. AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7)	Update Lane Number Set Next Steps (Not used. By default, the Sequencer API determines this functionality. If necessary, enable the automation and use it to override the next step behavior used by the Sequencer API.)

Terminology

This integration package supports BaseSpace Clarity LIMS v5.1 and later.

As of Clarity LIMS v5, the term user-defined field (UDF) has been replaced with custom field in the user interface. However, the API resource is still called UDF.

There are two types of custom fields:

- Master step fields—Configured on master steps. Master step fields only apply to the following:
  - The master step on which the fields are configured.
  - The steps derived from those master steps.
- Global fields—Configured on entities (eg, submitted sample, derived sample, measurement, etc.). Global fields apply to the entire Clarity LIMS system.

Protocol 1: Run Format (NovaSeq 6000 v3.7)

This protocol sets the Loading Workflow Type and allows the choice of the appropriate Flowcell Type and Final Loading Concentration (pM). After the protocol, a routing script sends the normalized libraries to either the NovaSeq Standard (NovaSeq 6000 v3.7) or the NovaSeq Xp (NovaSeq 6000 v3.7) protocol.

This protocol contains one step: Define Run Format (NovaSeq 6000 v3.7).

Step 1. Define Run Format (NovaSeq 6000 v3.7)

Step input: NTP (normalized libraries)

Step output: None

Set Next Steps Automation

Automatically triggered on exit of the Record Details screen, this automation completes the following actions:

- Sets the next step for samples to REMOVE:

```
nextStep = ::REMOVE::
```

- Calculates the Minimum Molarity using the following formula:

```
input.::Minimum Molarity (nM):: = (5 * input.::Final Loading Concentration
(pM)::)/1000
```

- Checks Normalized Molarity value. For samples with no Normalized Molarity value (ie, empty value, not including 0), it generates an error message informing that the field cannot be empty:

```
if (!input.hasValue(::Normalized Molarity (nM)::)) {
  fail(::The Normalized Molarity cannot be empty.::) ;
}
```

- Compares each sample Normalized Molarity value with the Minimum Molarity value. If the Normalized Molarity value is lower than the Minimum Molarity value, it sets the samples Loading Workflow Type to [Remove from workflow]. The automation also records a message in the Warning field for the sample:

```
else if (input.::Normalized Molarity (nM):: < input.::Minimum Molarity (nM)::) {
  input.::Warning:: = ::The Normalized Molarity is too low.:: ;
  input.::Loading Workflow Type:: = ::[Remove from workflow]:: ; }
else { input.::Warning:: = ::not applicable:: }
```

At this point there are two options:

- Correct the Normalized Molarity value on the Record Details screen. Edit the Loading Workflow Type field and set it to NovaSeq Standard or NovaSeq Xp, as applicable.
- Complete the protocol without correcting the Normalized Molarity value. In this case, those samples are removed from the workflow.

Routing Script Automation

Automatically triggered on exit of the step, this automation invokes the changeWorkflow script, which routes step inputs appropriately.

- Samples with Loading Workflow Type field value = NovaSeq Standard are routed to the NovaSeq 6000 v3.7 workflow. Then, the samples are queued for the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.7) step.
- Samples with Loading Workflow Type field value = NovaSeq Xp are routed to the NovaSeq 6000 v3.7 workflow. Then, the samples are queued for the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.7) step.

Default automation command line is as follows:

```
bash -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar
```


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```
-u {username} -p {password} -i {stepURI:v2} -l {compoundOutputFileLuid0}
script:changeWorkflow \
\
--FIELD_NAME 'Loading Workflow Type' \
--FIELD_VALUE 'NovaSeq Standard' \
--WORKFLOW 'NovaSeq 6000 v3.7' \
--STEP 'Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.7)' \
--INPUTS_OR_OUTPUTS 'INPUTS' \
\
--FIELD_NAME 'Loading Workflow Type' \
--FIELD_VALUE 'NovaSeq Xp' \--WORKFLOW 'NovaSeq 6000 v3.7' \
--STEP 'Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.7)' \
--INPUTS_OR_OUTPUTS 'INPUTS'"
```

Master Step Fields

The following table lists field configuration details defined on the Define Run Format (NovaSeq 6000 v3.7) master step.

Table 122 Define Run Format (NovaSeq 6000 v3.7) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Comment	Multiline Text		None
Flowcell Type	Text Dropdown	Required Field Custom Entries	Presets • SP • S1 • S2 • S4
Instruction	Text	Read Only	Default • Add Flowcell Type and Loading Workflow Type below
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp

Global Fields

The following table lists the global custom fields that are configured to display on the Define Run Format (NovaSeq 6000 v3.7) step.

Table 123 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
Adjusted Per Sample Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Final Loading Concentration (pM)	Numeric Dropdown	Required Field Custom Entries	Decimal places displayed = 0 Presets • 225 • 400
Flowcell Type	Text Dropdown	Required Field	Presets • SP • S1 • S2 • S4
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
Minimum Molarity (nM)	Numeric		Decimal places displayed = 2
Normalized Molarity (nM)	Numeric		Decimal places displayed = 2
Per Sample Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Warning	Text Dropdown	Read Only Custom Entries	The Normalized Molarity (nM) is too low. Not applicable

User Interaction

Refer to Run Define Run Format (NovaSeq 6000 v3.7) step section in *NovaSeq 6000 Integration v3.5.1 Validation and Troubleshooting* on page 429.

Protocol 2: NovaSeq Standard (NovaSeq 6000 v3.7)

Samples are routed to this protocol when their Loading Workflow Type value is set to NovaSeq Standard. Samples are pooled and added to a library tube in preparation for the NovaSeq run.

At the end of this protocol, a routing script sends the library tube to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7) protocol.

This protocol contains two steps:

- Step 1: Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.7)
- Step 2: Dilute and Denature (NovaSeq 6000 v3.7)

Step 1: Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.7)

In this step, libraries are placed manually into a single pool. Resuspension buffer and reagents are added.

Create only one pool per step.

Step input: NTP (normalized libraries)

Step output: Bulk pool

Validate Unique Indexes automation is not used. The core Clarity LIMS configuration for pooling handles this functionality.

Validate Inputs Flowcell Type and Single Pool Automation

Automatically triggered on exit of the Pooling screen, this automation completes the following actions:

- Checks that all samples in the pool have the same Flowcell Type assigned to them.
- Checks that only one pool has been created.
- Changes the value of error and logging messages to reference the type of pool passed as bulk pool.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar \
script:validate_flowcell_for_input_pools \
  -i {stepURI:v2} \
  -u {username} \
  -p {password} \
  -l {compoundOutputFileLuid1} \
  -validateSingleOutput true \
  -poolType bulk"
```

Calculate Volumes Automation

Automatically triggered when selecting the Calculate Volumes button on the Record Details screen, this automation completes the following actions:

- Calculates the number of samples in the pool.

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: + 1
```

- Sets the value of the Bulk Pool Volume (µl), and PhiX Volume (µl) field based on the selected Flowcell Type.

```
step.::PhiX Volume (ul):: = !step.hasValue(::% PhiX (2.5nM) Spike-In::) ? 0 :
step.::% PhiX (2.5nM) Spike-In::;
if (input.::Flowcell Type:: == ::SP::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 100;
step.::PhiX Volume (ul):: *= 0.6
};
if (input.::Flowcell Type:: == ::S1::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 100;
step.::PhiX Volume (ul):: *= 0.6
};
if (input.::Flowcell Type:: == ::S2::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 150;
step.::PhiX Volume (ul):: *= 0.9
};
if (input.::Flowcell Type:: == ::S4::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 310;
step.::PhiX Volume (ul):: *= 1.9
};
if (step.::PhiX Volume (ul):: == 0) {
step.::PhiX Volume (ul):: = ::::
};
```

- Calculates the Per Sample Volume (µl) to be added to the pool.

```
input.::Per Sample Volume (ul):: = ( ( (input.::Final Loading Concentration
(pM):: * 5 / 1000) / input.::Normalized Molarity (nM):: ) * step.::Bulk Pool
Volume (ul):: ) / step.::Number of Samples in Pool::
```

> For accurate pipetting of each sample in a pool, the Per Sample Volume (µl) value must be greater than or equal to the Minimum Per Sample Volume (µl). The default value (set at 5) can be edited.
>
> Assuming the default Minimum Per Sample Volume (µl) value of 5, completes the following steps for a given batch:
>
> 1. If the smallest Per Sample Volume (µl) value is less than 5, automatically assigns a value of 5 to the Adjusted Per Sample Volume (µl) field.
> 2. Adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch. This adjustment is based on the ratio used to increase the lowest value to 5.

- Calculates the Total Sample Volume (µl) field value.

```
step.::Total Sample Volume (µl):: = step.::Total Sample Volume (µl):: +
input.::Adjusted Per Sample Volume (µl)::
```

- If the Total Sample Volume is less than the Bulk Pool Volume, the automation calculates the RSB Volume (µl) field value.

```
if (step.::Total Sample Volume (ul):: >= step.::Bulk Pool Volume (ul)::)
{output.::RSB Volume (ul):: = 0}
else {output.::RSB Volume (ul):: = step.::Bulk Pool Volume (ul):: - step.::Total
Sample Volume (ul)::} ;
```

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs.

```
output.::Flowcell Type:: = input.::Flowcell Type:: ;
output.::Loading Workflow Type:: = input.::Loading Workflow Type:: ;
```

- Sets the Volume of Pool to Denature (µl) value and calculates the NaOH Volume (µl) and Tris-HCl Volume (µl) values based on the Flowcell Type:

```
if ( input.::Flowcell Type:: == ::SP:: ) { output.::Volume of Pool to Denature
(ul):: = 100 ; output.::NaOH Volume (ul):: = 25 ; output.::Tris-HCl Volume
(ul):: = 25 } ;
if ( input.::Flowcell Type:: == ::S1:: ) { output.::Volume of Pool to Denature
(ul):: = 100 ; output.::NaOH Volume (ul):: = 25 ; output.::Tris-HCl Volume
(ul):: = 25 } ;
if ( input.::Flowcell Type:: == ::S2:: ) { output.::Volume of Pool to Denature
(ul):: = 150 ; output.::NaOH Volume (ul):: = 37 ; output.::Tris-HCl Volume
(ul):: = 38 } ;
if ( input.::Flowcell Type:: == ::S4:: ) { output.::Volume of Pool to Denature
(ul):: = 310 ; output.::NaOH Volume (ul):: = 77 ; output.::Tris-HCl Volume
(ul):: = 78 }
```

- Uses the `NovaSeq_Standard_Bulk_Pool1.csv`, `NovaSeq_Standard_Bulk_Pool2.csv`, and `NovaSeq_Standard_Bulk_Pool3.csv` template files to generate a single CSV file containing information about the pool and the samples it contains. The generated file is available for download on the Step Setup screen of Dilute and Denature (NovaSeq 6000 v3.7).

```
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Standard_Bulk_
Pool1.csv \
-o 1.csv \
script:driver_file_generator \
```

```
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Standard_Bulk_
Pool2.csv \
-o 2.csv \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Standard_Bulk_
Pool3.csv \
-o 3.csv \
&& cat 1.csv 2.csv 3.csv > {compoundOutputFileLuid0}.csv
```

- Resets the Total Sample Volume (µl) and Number of Samples in Pool field values so that the automation is idempotent.

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: - 1;
step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: -
input.::Adjusted Per Sample Volume (ul)::
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, the following automation sets the next step for samples to ADVANCE. The samples advance to the Dilute and Denature (NovaSeq 6000 v3.7) step:

```
nextStep = ::ADVANCE::
```

Master Step Fields

The following table lists configuration details defined on the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.7) step.

Table 124 Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.7) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
% PhiX (2.5 nM) Spike-In	Numeric		Range = 0–100 Decimal places displayed = 0
Bulk Pool Volume (µl) (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 2

Field Name	Field Type	Options	Additional Options and Dropdown Items
Number of Flowcells to Sequence	Numeric	Required Field	Range = 1–10 Decimal places displayed = 0
Minimum Per Sample Volume (µl)	Numeric	Required Field	Decimal places displayed = 2 Default • 5
Number of Samples in Pool (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 0 Default • 0
PhiX Volume (µl) (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 2
Total Sample Volume (µl) (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 2 Default • 0

Global Fields

The following table lists the global custom fields that are configured to display on the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.7) step.

Table 125 Global Custom Fields Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
Flowcell Type	Text Dropdown	Required Field	Presets • SP • S1 • S2 • S4

Field Name	Field Type	Options	Additional Options and Dropdown Items
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
NaOH Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
RSB Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Tris-HCl Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Volume of Pool to Denature (µl) Used in Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.7) step only. Displays on Record Details screen and in the generated CSV file.	Numeric	Read Only	Decimal places displayed = 0

User Interaction

Refer to Run Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.7) step section in *NovaSeq 6000 Integration v3.5.1 Validation and Troubleshooting* on page 429.

Step 2: Dilute and Denature (NovaSeq 6000 v3.7)

In this step, the addition of NaOH, Tris-HCl, and Resuspension Buffer (RSB) denatures and dilutes the pooled samples. Manually place the pooled samples into the library tube for the NovaSeq run.

In addition, this step validates the run setup information and generates the sample sheet file.

Step input: Bulk pool

Step output: Library tube

Validate Single Input Automation

Automatically triggered at the beginning of the step, this automation checks that there is only one container input to the step.

```
script:validateSampleCount -min 1 -max 1
```

Validate Library Tube Barcode Automation

Automatically triggered on exit of the Placement screen, this automation completes the following actions:

- Validates the library tube barcode to ensure it conforms to the barcode mask [A-Z]{2}[0-9]{7}-[A-Z]{3}:

```
if (!output.container.name.matches(::[A-Z]{2}[0-9]{7}-[A-Z]{3}::)) {
fail(::Invalid Library Tube Barcode. Verify and try again.::)
}
```

- Copies Flowcell Type and Loading Workflow Type field values from step inputs to outputs.

```
output.::Flowcell Type:: = input.::Flowcell Type:: ;
output.::Loading Workflow Type:: = input.::Loading Workflow Type::
```

Validate Run Setup and Generate Sample Sheet Automation

The script definition portion of Validate Run Setup has been moved into the Validation Script custom field due to the 4000 character limit.

- If modification is required on Validate Run Setup script, perform the changes directly on the Validation Script custom field default value.
- The script definition portion of the Generate Sample Sheet remains untouched.

Triggered by a button on the Record Details screen, this automation completes the following actions:

- Copies the Flowcell Type from the step input to the Run Mode field (hidden):

```
step.::Run Mode:: = input.::Flowcell Type::
```

- Validates the parameters entered on the Record Details screen, which are used to set up the run and generate the sample sheet file. For more information, refer to the *Respond to Validation / Recipe Request Call from Instrument* section of *NovaSeq 6000 Run* on page 416.
  - Experiment Name can only contain alphanumeric, dash, or underscore characters. Spaces are not permitted.
  - When Workflow Type = No Index, both Index Read 1 and Index Read 2 must be zero.
  - When Workflow Type = Single Index, Index Read 1 must be greater than zero, and Index Read 2 must be zero.
  - When Workflow Type = Dual Index, both Index Read 1 and Index Read 2 must be greater than zero.

```
if(!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) {
fail(::Experiment Name contains prohibited characters. Allowed characters are:
a-z, A-Z, 0-9, -, and _::)
```

```
};
if (step.::Workflow Type:: == ::No Index::) {
if (step.::Index Read 1:: != 0 || step.::Index Read 2:: != 0) {
fail(::Index Read 1 and Index Read 2 must be 0 if No Index is selected.::)
}
} else if (step.::Workflow Type:: == ::Single Index::) {
if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) {
fail(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if Single
Index is selected.::)
}
} else {
if (step.::Index Read 1:: == 0 || step.::Index Read 2:: == 0) {
fail(::Index Read 1 and Index Read 2 must be greater than 0 if Dual Index is
selected.::)
}
};
```

- Checks the Paired End and Read 2 Cycles field values.
  - If Paired End = True and Read 2 Cycles value is zero, generates an error.
  - If Paired End = False, Read 2 Cycles must be zero and UMI - Read 2 Length and UMI - Read 2 Start From Cycle must be empty.

```
if (step.::Paired End::.toBoolean()) {
if (step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) {
fail(::Read 1 Cycles and Read 2 Cycles must be greater than 0 if Paired End is
True.::)
}
} else {
if (step.::Read 2 Cycles:: != 0) {
fail(::Read 2 Cycles must be 0 if Paired End is False.::)
};
if (step.hasValue(::UMI - Read 2 Length::) || step.hasValue(::UMI - Read 2 Start
From Cycle::)) {
fail(::UMI - Read 2 Length and UMI - Read 2 Start From Cycle cannot be defined
if Paired End is False.::)
}
};
```

- Checks the Flowcell field value.
  - If Flowcell Type value is not SP, checks that the values of Read 1 Cycles and Read 2 Cycles are each 151 or less. If the value is greater than 151, an error message is generated.

```
if (input.::Flowc ell Type::!=::SP:: && step.::Read 1 Cycles:: > 151) {
```

```
fail(::Read 1 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
};
if (input.::Flowcell Type::!=::SP:: && step.::Read 2 Cycles:: > 151) {
fail(::Read 2 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
};
```

- Validates UMI Settings.
  - If UMI - Read 1 Length has value, then UMI - Read 1 Start From Cycle must have value.
  - If UMI - Read 1 Length has no value, then UMI - Read 1 Start From Cycle must not have value.
  - If UMI - Read 2 Length has value, then UMI - Read 2 Start From Cycle must have value.
  - If UMI - Read 2 Length has no value, then UMI - Read 2 Start From Cycle must not have value.
  - If UMI - Read 1 Length has no value, then UMI - Read 2 Length must not have value.
  - If UMI - Read 1 Start From Cycle has no value, then UMI - Read 2 Start From Cycle must not have value.

```
if (step.hasValue(::UMI - Read 1 Length::) && !step.hasValue(::UMI - Read 1
Start From Cycle::)) {
fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 1
Length is greater than 0.::)
};
if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 1
Start From Cycle::)) {
fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 1 Start From
Cycle is greater than 0.::)
};
if (step.hasValue(::UMI - Read 2 Length::) && !step.hasValue(::UMI - Read 2
Start From Cycle::)) {
fail(::UMI - Read 2 Start From Cycle must be greater than 0 if UMI - Read 2
Length is greater than 0.::)
};
if (!step.hasValue(::UMI - Read 2 Length::) && step.hasValue(::UMI - Read 2
Start From Cycle::)) {
fail(::UMI - Read 2 Length must be greater than 0 if UMI - Read 2 Start From
Cycle is greater than 0.::)
};
if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 2
Length::)) {
fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 2 Length is
greater than 0.::)
};
```


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```
if (!step.hasValue(::UMI - Read 1 Start From Cycle::) && step.hasValue(::UMI -
Read 2 Start From Cycle::)) {
fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 2
Start From Cycle is greater than 0.::)
};
```

- Validate Analysis Software Version to ensure it is a valid version string.

```
if (step.hasValue(::Analysis Software Version::) && !step.::Analysis Software
Version::.matches(::^(\\d+)(\\.\\d+)*$::)) {
fail(::Analysis Software Version contains prohibited characters. Allowed
characters are 0-9 and period. It shall start and end with numbers and separated
by single period e.g. 3.8.4::)
};
```

- Validate Override Cycles value to contain only Y, N, I, U, 0-9, and semicolon characters.

```
if (step.hasValue(::Override Cycles::) && !step.::Override Cycles::.matches(::
[YNIU0-9;]+::)) {
fail(::Override Cycles contains prohibited characters. Allowed characters are:
Y, N, I, U, 0-9 and ;. Example: N1Y150;I8;I7N1;Y141U10.::)
};
```

- Outputs the Settings Header in sample sheet when UMI - Read 1 Length or UMI - Read 2 Length have value.

```
step.::Settings Header:: = step.hasValue(::UMI - Read 1 Length::) ||
step.hasValue(::UMI - Read 2 Length::) ? ::[Settings]:: : ::::;
```

- Sets the next step for samples to REMOVE.

```
nextStep = ::REMOVE::
```

- Generates the sample sheet and attaches it to the step. For more information, refer to *Sample Sheet Generation* on page 582. Sample sheet template used depends on the selected value of Samplesheet Format and/or Reverse Complement Workflow.

```
&& if [ '{udf:Samplesheet Format}' == 'V2' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Samplesheet_v2.csv'; \
elif [ '{udf:Reverse Complement Workflow}' == 'true' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Reverse_Complement_Samplesheet.csv'; \
elif [ '{udf:Reverse Complement Workflow}' == 'false' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Samplesheet.csv'; \
else echo 'UDF Reverse Complement Workflow is empty or undefined.'; exit -1; fi
\
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} \
script:driver_file_generator \
```

```
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/$templateFilename \
-o {compoundOutputFileLuid0}.csv \
-q true \
-destLIMSID {compoundOutputFileLuid0} \
-l {compoundOutputFileLuid1}"
```

Routing Script Automation

Automatically triggered on exit of the step, this automation invokes the changeWorkflow script. This script routes step outputs to the NovaSeq 6000 v3.7 workflow and queues them for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7) step.

Default automation command line is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar
-u {username} -p {password} -i {stepURI:v2} -l {compoundOutputFileLuid2}
script:changeWorkflow \
--FIELD_NAME 'N/A' \
--FIELD_VALUE 'N/A' \
--WORKFLOW 'NovaSeq 6000 v3.7' \
--STEP 'AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7)' \
--INPUTS_OR_OUTPUTS 'OUTPUTS'"
```

Master Step Fields

The following table lists field configuration details defined on the Dilute and Denature (NovaSeq 6000 v3.7) step. These fields are required for sample sheet and JSON file generation.

Table 126 Dilute and Denature (NovaSeq 6000 v3.7) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Analysis Software Version	Text		
BaseSpace Sequence Hub Configuration	Text Dropdown	Required Field	Presets • Not Used • Run Monitoring Only • Run Monitoring and Storage
Custom Recipe Path	Text		Not applicable

Field Name	Field Type	Options	Additional Options and Dropdown Items
Experiment Name	Text	Required Field	Not applicable
Index Read 1	Numeric Dropdown	Required Field Custom Entries	Range = 0–20 Decimal places displayed = 0 Presets • 0 • 6 • 8
Index Read 2	Numeric Dropdown	Required Field Custom Entries	Range = 0–20 Decimal places displayed = 0 Presets • 0 • 6 • 8
Output Folder	Text	Required Field	Not applicable
Override Cycles	Text		
Paired End	Text Dropdown	Required Field	Presets • True • False
Read 1 Cycle	Numeric Dropdown	Required Field Custom Entries	Range = 1–251 Decimal places displayed = 0 Presets • 251* • 151 • 101 • 51 *Value of 251 is only supported for SP flow cell type. For all other cell types, maximum value is 151.

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Field Name	Field Type	Options	Additional Options and Dropdown Items
Read 2 Cycle	Numeric Dropdown	Required Field Custom Entries	Range = 0–251 Decimal places displayed = 0 Presets • 251* • 151 • 101 • 51 *Value of 251 is only supported for SP flow cell type. For all other cell types, maximum value is 151.
Reverse Complement Workflow	Toggle Switch	Required Field	Default • Yes
Run Mode (Not displayed in user interface)	Text Dropdown	Read Only	Presets • SP • S1 • S2 • S4
Samplesheet Format	Text Dropdown	Required Field	Presets • V1 (default) • V2
Setting Header (not displayed in user interface)	Text	Read Only	Not applicable
UMI—Read 1 Length	Numeric		Range = 1
UMI—Read 1 Start From Cycle	Numeric		Range = 1
UMI—Read 2 Length	Numeric		Range = 1
UMI—Read 2 Start From Cycle	Numeric		Range = 1
Use Custom Index Read 1 Primer	Toggle Switch		Default • None Set

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Field Name	Field Type	Options	Additional Options and Dropdown Items
Use Custom Read 1 Primer	Toggle Switch		Default • None Set
Use Custom Read 2 Primer	Toggle Switch		Default • None Set
Use Custom Recipe	Toggle Switch	Required Field	Default • No
Validation Script	Multiline Text	Required Field Read Only	Default value is provided in the drop-down section that follows the table. Do not remove this field as it is used by Validate Run Setup and Generate Sample Sheet automation script.
Workflow	Text	Read Only	Default • GenerateFASTQ
Workflow Type	Text Dropdown	Required Field	Presets • No Index • Single Index • Dual Index • Custom

Validation Script

```
step.::Run Mode:: = input.::Flowcell Type::; if (!step.::Experiment
Name::.matches(::[a-zA-Z0-9-_]+::)) { fail(::Experiment Name contains prohibited
characters. Allowed characters are: a-z, A-Z, 0-9, -, and _::) }; if
(step.::Workflow Type:: == ::No Index::) { if (step.::Index Read 1:: != 0 ||
step.::Index Read 2:: != 0) { fail(::Index Read 1 and Index Read 2 must be 0 if
No Index is selected.::) } } else if (step.::Workflow Type:: == ::Single
Index::) { if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) { fail
(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if Single
Index is selected.::) } } else { if (step.::Index Read 1:: == 0 || step.::Index
```

```
Read 2:: == 0) { fail(::Index Read 1 and Index Read 2 must be greater than 0 if
Dual Index is selected.::) } }; if (step.::Paired End::.toBoolean()) { if
(step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) { fail(::Read 1
Cycles and Read 2 Cycles must be greater than 0 if Paired End is True.::) } }
else { if (step.::Read 2 Cycles:: != 0) { fail(::Read 2 Cycles must be 0 if
Paired End is False.::) }; if (step.hasValue(::UMI - Read 2 Length::) ||
step.hasValue(::UMI - Read 2 Start From Cycle::)) { fail(::UMI - Read 2 Length
and UMI - Read 2 Start From Cycle cannot be defined if Paired End is False.::) }
}; if (input.::Flowcell Type:: != ::SP:: && step.::Read 1 Cycles:: > 151) { fail
(::Read 1 Cycles must not be larger than 151 if it is not SPrime Flowcell::) };
if (input.::Flowcell Type:: != ::SP:: && step.::Read 2 Cycles:: > 151) { fail
(::Read 2 Cycles must not be larger than 151 if it is not SPrime Flowcell::) };
if (step.hasValue(::UMI - Read 1 Length::) && !step.hasValue(::UMI - Read 1
Start From Cycle::)) { fail(::UMI - Read 1 Start From Cycle must be greater than
0 if UMI - Read 1 Length is greater than 0.::) }; if (!step.hasValue(::UMI -
Read 1 Length::) && step.hasValue(::UMI - Read 1 Start From Cycle::)) { fail
(::UMI - Read 1 Length must be greater than 0 if UMI - Read 1 Start From Cycle
is greater than 0.::) }; if (step.hasValue(::UMI - Read 2 Length::) &&
!step.hasValue(::UMI - Read 2 Start From Cycle::)) { fail(::UMI - Read 2 Start
From Cycle must be greater than 0 if UMI - Read 2 Length is greater than 0.::)
}; if (!step.hasValue(::UMI - Read 2 Length::) && step.hasValue(::UMI - Read 2
Start From Cycle::)) { fail(::UMI - Read 2 Length must be greater than 0 if UMI
- Read 2 Start From Cycle is greater than 0.::) }; if (!step.hasValue(::UMI -
Read 1 Length::) && step.hasValue(::UMI - Read 2 Length::)) { fail(::UMI - Read
1 Length must be greater than 0 if UMI - Read 2 Length is greater than 0.::) };
if (!step.hasValue(::UMI - Read 1 Start From Cycle::) && step.hasValue(::UMI -
Read 2 Start From Cycle::)) { fail(::UMI - Read 1 Start From Cycle must be
greater than 0 if UMI - Read 2 Start From Cycle is greater than 0.::) }; if
(step.hasValue(::Analysis Software Version::) && !step.::Analysis Software
Version::.matches(::^(\\d+)(\\.\\d+)*$::)) { fail(::Analysis Software Version
contains prohibited characters. Allowed characters are 0-9 and period. It shall
start and end with numbers and separated by single period e.g. 3.8.4::) }; if
(step.hasValue(::Override Cycles::) && !step.::Override Cycles::.matches(::
[YNIU0-9;]+::)) { fail(::Override Cycles contains prohibited characters. Allowed
characters are: Y, N, I, U, 0-9 and ;. Example: N1Y150;I8;I7N1;Y141U10.::) };
step.::Settings Header:: = step.hasValue(::UMI - Read 1 Length::) ||
step.hasValue(::UMI - Read 2 Length::) ? ::[Settings]:: : ::::; nextStep =
::REMOVE::
```


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Global Fields

The following table lists the global custom fields that are configured to display on the Dilute and Denature (NovaSeq 6000 v3.7) step.

Table 127 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
Flowcell Type	Text Dropdown	Required Field	Presets • SP • S1 • S2 • S4
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User Interaction

Refer to Run Dilute and Denature (NovaSeq 6000 v3.7) step section in *NovaSeq 6000 Integration v3.5.1 Validation and Troubleshooting* on page 429.

Protocol 3: NovaSeq Xp (NovaSeq 6000 v3.7)

Samples are routed to this protocol when their Loading Workflow Type value is set to NovaSeq Xp.

Samples are pooled and added to lanes on the NovaSeq flow cell type selected in the Define Run Format (NovaSeq 6000 v3.7) step. At the end of this protocol, the flow cell is sent to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7) protocol.

This protocol contains the following three steps:

- Step 1: Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.7)
- Step 2: Dilute, Denature, and ExAmp (NovaSeq 6000 v3.7)
- Step 3: Load to Flowcell (NovaSeq 6000 v3.7)

Step 1: Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.7)

Manually place libraries into a pool.

Create only one pool per step.

Step input: NTP (normalized libraries)

Step output: Bulk pool

Validate Unique Indexes automation is not used. The core Clarity LIMS configuration for pooling handles this functionality.

Validate Inputs Flowcell Type and Single Pool Automation

Automatically triggered on exit of the Pooling screen, this automation completes the following actions:

- Checks that all samples in the pool have the same Flowcell Type assigned to them.
- Checks that only one pool has been created.
- Changes the value of error and logging messages to reference the type of pool passed as 'bulk' pool.

```
/opt/gls/clarity/bin/java \
-jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar \
script:validate_flowcell_for_input_pools \
-i {stepURI:v2} \
-u {username} \
-p {password} \
-l {compoundOutputFileLuid1} \
-validateSingleOutput true \
-poolType bulk"
```

Calculate Volumes Automation

Triggered when the Calculate Volumes button is selected on the Record Details screen, this automation completes the following actions:

- Calculates the number of samples in the pool.

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: + 1
```

- Sets the value of the Bulk Pool Volume (µl) and PhiX Volume (µl) field, based on the selected Flowcell Type.

```
step.::PhiX Volume (ul):: = !step.hasValue(::% PhiX (0.25nM) Spike-In::) ? 0 :
step.::% PhiX (0.25nM) Spike-In::;
if (input.::Flowcell Type:: == ::SP::) {
step.::Bulk Pool Volume (ul):: = step.'Number of Lanes to Sequence' * 18;
step.::PhiX Volume (ul):: *= 0.7;
};
if (input.::Flowcell Type:: == ::S1::) {
step.::Bulk Pool Volume (ul):: = step.'Number of Lanes to Sequence' * 18;
step.::PhiX Volume (ul):: *= 0.7;
};
```

```
if (input.::Flowcell Type:: == ::S2::) {
step.::Bulk Pool Volume (ul):: = step.'Number of Lanes to Sequence' * 22;
step.::PhiX Volume (ul):: *= 0.8;
};
if (input.::Flowcell Type:: == ::S4::) {
step.::Bulk Pool Volume (ul):: = step.'Number of Lanes to Sequence' * 30;
step.::PhiX Volume (ul):: *= 1.1;
};
if (step.::PhiX Volume (ul):: == 0) {
step.::PhiX Volume (ul):: = ::::;
};
```

- Calculates the Per Sample Volume (µl) to be added to the pool.

```
input.::Per Sample Volume (µl)::= (((input.::Final Loading Concentration (pM)::*
5/ 1000) / input.::Normalized Molarity (nM)::) * step.::Bulk Pool Volume (µl)::)
/ step.::Number of Samples in Pool::
```

> For accurate pipetting of each sample in a pool, the Per Sample Volume (µl) value must be greater than or equal to the Minimum Per Sample Volume (µl). The default value (set at 5) can be edited.
>
> Assuming the default Minimum Per Sample Volume (µl) value of 5, completes the following steps for a given batch:
>
> 1. If the smallest Per Sample Volume (µl) value is less than 5, automatically assigns a value of 5 to the sample Adjusted Per Sample Volume (µl) field.
> 2. Adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch. This adjustment is based on the ratio used to increase the lowest value to 5.

- Calculates the Total Sample Volume (µl) field value.

```
step.::Total Sample Volume (µl):: = step.::Total Sample Volume (µl):: +
input.::Adjusted Per Sample Volume (µl)::
```

- If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) field value.

```
if (step.::Total Sample Volume (µl):: >= step.::Bulk Pool Volume (µl)::)
{output.::RSB Volume (µl):: = 0} else {output.::RSB Volume (µl):: = step.::Bulk
Pool Volume (µl):: - step.::Total Sample Volume (µl)::} ;
```

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs.

```
output.::Flowcell Type::= input.::Flowcell Type::;output.::Loading Workflow
Type::= input.::Loading Workflow Type::;
```

- Uses the `NovaSeq_Xp_Bulk_Pool.csv` and `NovaSeq_Xp_Bulk_Pool2.csv` template files to generate a single CSV file containing information about the bulk pool and the samples it contains. The generated file is available for download on the Step Setup screen of the Dilute, Denature, and ExAmp (NovaSeq 6000 v3.7) step.

```
&& /opt/gls/clarity/bin/java \-jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar \
  -i {stepURI:v2} \
  -u {username} \
  -p {password} \
  -l {compoundOutputFileLuid1} \ script:driver_file_generator \
  -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_Bulk_
Pool.csv \
  -o {compoundOutputFileLuid0}.csv \ script:driver_file_generator \
  -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_Bulk_
Pool2.csv \
  -o append.csv \
&& cat append.csv >> {compoundOutputFileLuid0}.csv"
```

- Resets the Total Sample Volume (µl) and Number of Samples in Pool field values so that the automation is idempotent.

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: - 1;
step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: -
input.::Adjusted Per Sample Volume (ul)::
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation completes the following actions:

- Copies the Flowcell Type values from the step inputs to the step outputs.
- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol.

```
output.::Flowcell Type:: = input.::Flowcell Type:: ; nextStep = ::ADVANCE::
```

Master Step Fields

The following table lists field configuration details defined on the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.7) step.

Table 128 Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.7) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
% PhiX (0.25nM) Spike-in	Numeric		Range = 0–100
Bulk Pool Volume (µl) (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 2
Minimum Per Sample Volume (µl)	Numeric	Required Field	Decimal places displayed = 2 Default • 5
Number of Lanes to Sequence	Numeric	Required Field	Decimal places displayed = 0
Number of Samples in Pool (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 0 Default • 0
PhiX Volume (µl) (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 2
Total Sample Volume (µl) (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 0 Default • 0

Global Fields

The following table lists the global custom fields that are configured to display on the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.7) step.

Table 129 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
RSB Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Flowcell Type	Text Dropdown	Required Field	Presets • SP • S1 • S2 • S4
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User Interaction

Refer to Run Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.7) step section in *NovaSeq 6000 Integration v3.5.1 Validation and Troubleshooting* on page 429.

Step 2: Dilute, Denature and ExAmp (NovaSeq 6000 v3.7)

In this step, the addition of DPX, NaOH, Tris-HCl, and RSB denatures and dilutes the pooled samples. Manually create working pools based on the number of lanes that you want to sequence.

Step input: Bulk pool

Step output: Working pool - variable number, choose how many working pools to create per bulk pool

Validate Inputs Flowcell Type Automation

Automatically triggered at the beginning of the step, this automation invokes the `validate_flowcell_for_input_pools` script. This script completes the following actions:

- Checks the inputs to the step and validates that the Flowcell Type field is set to a valid value (SP, S1, S2, or S4). The automation also validates that all inputs have the same value for this field.
- The container type selected matches the value in the Flowcell Type field.

```
bash -l -c "/opt/gls/clarity/bin/java \
-jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar \
script:validate_flowcell_for_input_pools \
-i {stepURI:v2} \
```

```
-u {username} \
-p {password} \
-l {compoundOutputFileLuid1}"
```

Calculate Volumes Automation

Automatically triggered on entry to of the Record Details screen, this automation sets the value of the following fields based on the Flowcell Type:

- BP Aliquot Volume (µl)
- NaOH Volume (µl)
- Tris-HCl Volume (µl)
- DPX1 Volume (µl)
- DPX2 Volume (µl)
- DPX3 Volume (µl)
- Mastermix per Lane (µl)

```
if ( input.::Flowcell Type:: == ::SP:: ) {
output.::BP Aliquot Volume (ul):: = 18 ;
output.::NaOH Volume (ul):: = 4;
output.::Tris-HCl Volume (ul):: = 5;
step.::DPX1 Volume (ul):: = 126 ;
step.::DPX2 Volume (ul):: = 18 ;
step.::DPX3 Volume (ul):: = 66 ;
output.::Mastermix per Lane (ul):: = 63
};
if (input.::Flowcell Type:: == ::S1::) {
output.::BP Aliquot Volume (ul):: = 18;
output.::NaOH Volume (ul):: = 4;
output.::Tris-HCl Volume (ul):: = 5;
step.::DPX1 Volume (ul):: = 126;
step.::DPX2 Volume (ul):: = 18;
step.::DPX3 Volume (ul):: = 66;
output.::Mastermix per Lane (ul):: = 63
};
if (input.::Flowcell Type:: == ::S2::) {
output.::BP Aliquot Volume (ul):: = 22;
output.::NaOH Volume (ul):: = 5;
output.::Tris-HCl Volume (ul):: = 6;
step.::DPX1 Volume (ul):: = 126;
```


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```
step.::DPX2 Volume (ul):: = 18;
step.::DPX3 Volume (ul):: = 66;
output.::Mastermix per Lane (ul):: = 77
};
if (input.::Flowcell Type:: == ::S4::) {
output.::BP Aliquot Volume (ul):: = 30;
output.::NaOH Volume (ul):: = 7;
output.::Tris-HCl Volume (ul):: = 8;
step.::DPX1 Volume (ul):: = 315;
step.::DPX2 Volume (ul):: = 45;
step.::DPX3 Volume (ul):: = 165;
output.::Mastermix per Lane (ul):: = 105
};
```

The automation also completes the following actions:

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs.

```
output.::Flowcell Type::= input.::Flowcell Type::;
output.::Loading Workflow Type::= input.::Loading Workflow Type::;
```

- Uses the `NovaSeq_Xp_Working_Pool.csv` and `NovaSeq_Xp_Working_Pool2.csv` template files to generate a single CSV file. This file contains information about the DPX volume and the volume of BP Aliquot, Mastermix, NaOH, and Tris-HCl to add per working pool. The generated file is available for download on the Step Setup screen of the Load to Flowcell (NovaSeq 6000 v3.7) step.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_Working_
Pool.csv \
-o {compoundOutputFileLuid0}.csv \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_Working_
Pool2.csv \
-o append.csv \
&& cat append.csv >> {compoundOutputFileLuid0}.csv"
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation completes the following actions:

- Copies the Flowcell Type values from the step inputs to the step outputs.
- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ; nextStep = ::ADVANCE::
```

Master Step Fields

The following table lists field configuration details defined on the Load to Flowcell (NovaSeq 6000 v3.7) step. A script sets these field values. The values are not editable while running the step.

Table 130 Dilute, Denature, and ExAmp (NovaSeq v3.7) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
DPX1 Volume (µl)	Numeric	Read Only	Decimal places displayed = 0
DPX2 Volume (µl)	Numeric	Read Only	Decimal places displayed = 0
DPX3 Volume (µl)	Numeric	Read Only	Decimal places displayed = 0

Global Fields

The following table lists the global custom fields that are configured to display on the Dilute, Denature, and ExAmp (NovaSeq 6000 v3.7) step.

Table 131 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
BP Aliquot Volume (µl)	Numeric	Read Only	Decimal places displayed = 0
Flowcell Type	Text Dropdown	Required Field	Presets • SP • S1 • S2 • S4

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Field Name	Field Type	Options	Additional Options and Dropdown Items
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
Mastermix per Lane (µl)	Numeric	Read Only	Decimal places displayed = 0
NaOH Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Tris-HCl Volume (µl)	Numeric	Read Only	Decimal places displayed = 2

User Interaction

Refer to Run Dilute, Denature & ExAmp (NovaSeq 6000 v3.7) step section in *NovaSeq 6000 Integration v3.5.1 Validation and Troubleshooting* on page 429.

Step 3: Load to Flowcell (NovaSeq 6000 v3.7)

In this step, scan the flow cell barcode into the Clarity LIMS. Then, manually place the working pools into the lanes of the flow cell for the NovaSeq run. This step validates the run setup information and generates the sample sheet file.

Step input: Working pool

Step output: Flow cell (output containers: SP, S1, and S2 with 2 lanes, and S4 with 4 lanes)

Validate Inputs and Selected Container Automation

Automatically triggered at the beginning of the step, this automation invokes the `validate_flowcell_for_input_pools` and `validateSelectedContainer` scripts. These scripts validate the step inputs and the container selected by the user, as follows.

- Checks that the Flowcell Type field has been set to a valid value (SP, S1, S2, or S4) and that all inputs have the same value.
- Checks that the number of outputs matches the number of lanes on the selected flow cell type. If validation fails, an error message informs that the number of working pools does not match the number of lanes available on the flow cell.
  The following table defines the number of samples allowed:

Flow Cell Type	Number of Working Pools
SP	2
S1	2
S2	2
S4	4

- Checks that the container type selected on entry to the Placement screen matches the value in the Flowcell Type field. If validation fails, an error message displays.

```
bash -l -c "/opt/gls/clarity/bin/java \
-jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar
\script:validate_flowcell_for_input_pools \
  -i {stepURI:v2} \
  -u {username} \
  -p {password} \
  -l {compoundOutputFileLuid1} \
  -validateSelectedContainer true"
```

Validate Flowcell Barcode Automation

Automatically triggered on exit of the Placement screen, this automation validates the flow cell barcode scanned into the Clarity LIMS by the user, using the following logic:

```
if (input.::Flowcell Type:: == ::SP:: && !output.container.name.matches( ::[A-
Z0-9]{5}DR[A-Z0-9]{2}:: ) ) {
fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: )
};
if (input.::Flowcell Type:: == ::S1:: && !output.container.name.matches( ::[A-
Z0-9]{5}DR[A-Z0-9]{2}:: ) ) {
fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: )
};
if (input.::Flowcell Type:: == ::S2:: && !output.container.name.matches( ::[A-
Z0-9]{5}DM[A-Z0-9]{2}:: ) ) {
fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: )
};
if (input.::Flowcell Type:: == ::S4:: && !output.container.name.matches( ::[A-
Z0-9]{5}DS[A-Z0-9]{2}:: ) ) {
fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: )
};
```

- Copies the Flowcell Type and Loading Workflow Type field values from step inputs to outputs:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ;
```

```
output.::Loading Workflow Type:: = input.::Loading Workflow Type::
```

Validate Run Setup and Generate Sample Sheet Automation

The script definition portion of Validate Run Setup has been moved into the Validation Script custom field due to the 4000 character limitation.

- If modification is required to the Validate Run Setup script, perform the changes directly on Validation Script custom field default value.
- The script definition portion of Generate Sample Sheet is unchanged.

Automatically triggered by selecting a button on the Record Details screen, this automation completes the following actions:

- Copies the Flowcell Type from the step input to the Run Mode field (hidden):

```
step.::Run Mode:: = input.::Flowcell Type::
```

- Validates the parameters entered on the Record Details screen, which are used to set up the run and generate the sample sheet file. For more information, refer to the *Respond to Validation / Recipe Request Call from Instrument* section of *NovaSeq 6000 Run* on page 416.
  - Experiment Name can only contain alphanumeric, dash, or underscore characters. Spaces are not permitted.
  - When Workflow Type = No Index, both Index Read 1 and Index Read 2 must be zero.
  - When Workflow Type = Single Index, Index Read 1 must be greater than zero and Index Read 2 must be zero.
  - When Workflow Type = Dual Index, both Index Read 1 and Index Read 2 must be greater than zero.

```
if(!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) {
fail(::Experiment Name contains prohibited characters. Allowed characters
are: a-z, A-Z, 0-9, -, and _::)
};
if (step.::Workflow Type:: == ::No Index::) {
if (step.::Index Read 1:: != 0 || step.::Index Read 2:: != 0) {
fail(::Index Read 1 and Index Read 2 must be 0 if No Index is selected.::)
}
} else if (step.::Workflow Type:: == ::Single Index::) {
if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) {
fail(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if
Single Index is selected.::)
}
} else {
if (step.::Index Read 1:: == 0 || step.::Index Read 2:: == 0) {
```

```
fail(::Index Read 1 and Index Read 2 must be greater than 0 if Dual Index is
selected.::)
}
};
```

- Checks the Paired End and Read 2 Cycles field values.
  - If Paired End = True and Read 2 Cycles value is 0, an error displays.
  - If Paired End = False, Read 2 Cycles must be zero and UMI - Read 2 Length and UMI - Read 2 Start From Cycle must be empty.

```
if (step.::Paired End::.toBoolean()) {
if (step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) {
fail(::Read 1 Cycles and Read 2 Cycles must be greater than 0 if Paired End
is True.::)
}
} else {
if (step.::Read 2 Cycles:: != 0) {
fail(::Read 2 Cycles must be 0 if Paired End is False.::)
};
if (step.hasValue(::UMI - Read 2 Length::) || step.hasValue(::UMI - Read 2
Start From Cycle::)) {
fail(::UMI - Read 2 Length and UMI - Read 2 Start From Cycle cannot be
defined if Paired End is False.::)
}
};
```

- Checks the Flowcell field value.

  If Flowcell Type value is not SP, checks that the value of both Read 1 Cycles and Read 2 Cycles is 151 or less. If value is greater than 151, the automation generates an error message.

```
if (input.::Flowcell Type::!=::SP:: && step.::Read 1 Cycles:: > 151) {
fail(::Read 1 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
}
if (input.::Flowcell Type::!=::SP:: && step.::Read 2 Cycles:: > 151) {
fail(::Read 2 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
}
```

- Validates UMI Settings:
  - If UMI - Read 1 Length has value, UMI - Read 1 Start From Cycle must have value.
  - If UMI - Read 1 Length has no value, UMI - Read 1 Start From Cycle must not have value.
  - If UMI - Read 2 Length has value, UMI - Read 2 Start From Cycle must have value.
  - If UMI - Read 2 Length has no value, UMI - Read 2 Start From Cycle must not have value.
  - If UMI - Read 1 Length has not value, UMI - Read 2 Length must not have value.

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- If UMI - Read 1 Start From Cycle has no value, UMI - Read 2 Start From Cycle must not have value.

```
if (step.hasValue(::UMI - Read 1 Length::) && !step.hasValue(::UMI - Read 1 Start From Cycle::)) {
fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 1 Length is greater than 0.::)
};
if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 1 Start From Cycle::)) {
fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 1 Start From Cycle is greater than 0.::)
};
if (step.hasValue(::UMI - Read 2 Length::) && !step.hasValue(::UMI - Read 2 Start From Cycle::)) {
fail(::UMI - Read 2 Start From Cycle must be greater than 0 if UMI - Read 2 Length is greater than 0.::)
};
if (!step.hasValue(::UMI - Read 2 Length::) && step.hasValue(::UMI - Read 2 Start From Cycle::)) {
fail(::UMI - Read 2 Length must be greater than 0 if UMI - Read 2 Start From Cycle is greater than 0.::)
};
if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 2 Length::)) {
fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 2 Length is greater than 0.::)
};
if (!step.hasValue(::UMI - Read 1 Start From Cycle::) && step.hasValue(::UMI - Read 2 Start From Cycle::)) {
fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 2 Start From Cycle is greater than 0.::)
};
```

- Validates Analysis Software Version to make sure it is a valid version string.

```
if (step.hasValue(::Analysis Software Version::) && !step.::Analysis Software Version::.matches(::^(\\d+)(\\.\\d+)*$::)) {
fail(::Analysis Software Version contains prohibited characters. Allowed characters are 0-9 and period. It shall start and end with numbers and separated by single period e.g. 3.8.4::)
};
```


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- Checks Override Cycles field value. This value can contain only Y, N, I, U, 0–9, and semicolon characters.

```
if (step.hasValue(::Override Cycles::) && !step.::Override Cycles::.matches(::
[YNIU0-9;]+::)) {
fail(::Override Cycles contains prohibited characters. Allowed characters are:
Y, N, I, U, 0-9 and ;. Example: N1Y150;I8;I7N1;Y141U10.::)
};
```

- Outputs the Settings Header in sample sheet when UMI - Read 1 Length or UMI - Read 2 Length have values.

```
step.::Settings Header:: = step.hasValue(::UMI - Read 1 Length::) ||
step.hasValue(::UMI - Read 2 Length::) ? ::[Settings]:: : ::::;
```

- Sets the next step for samples to ADVANCE, which advances to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7) step.

```
nextStep = ::ADVANCE::
```

- Generates the sample sheet and attaches it to the step. The sample sheet template depends on the selected value for Samplesheet Format and/or Reverse Complete Workflow. For more information, refer to *Sample Sheet Generation* on page 415.

```
&& if [ '{udf:Samplesheet Format}' == 'V2' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Samplesheet_v2.csv'; \
elif [ '{udf:Reverse Complement Workflow}' == 'true' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Reverse_Complement_Samplesheet.csv'; \
elif [ '{udf:Reverse Complement Workflow}' == 'false' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Samplesheet.csv'; \
else echo 'UDF Reverse Complement Workflow is empty or undefined.'; exit -1; fi
\
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/$templateFilename \
-o {compoundOutputFileLuid0}.csv \
-q true \
-destLIMSID {compoundOutputFileLuid0} \
-l {compoundOutputFileLuid1}"
```

Master Step Fields

The following table lists the field configuration details defined on the Load to Flowcell (NovaSeq 6000 v3.7) step.

Table 132 Load to Flowcell (NovaSeq 6000 v3.7) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Analysis Software Version	Text		
BaseSpace Sequence Hub Configuration	Text Dropdown	Required Field	Presets • Not Used • Run Monitoring Only • Run Monitoring and Storage
Custom Recipe Path	Text		
Experiment Name	Text	Required Field	
Index Read 1	Numeric Dropdown	Required Field Custom Entries	Range = 0–20 Decimal places displayed = 0 Presets • 0 • 6 • 8
Index Read 2	Numeric Dropdown	Required Field Custom Entries	Range = 0–20 Decimal places displayed = 0 Presets • 0 • 6 • 8
Library Tube Barcode	Text	Required Field	
Output Folder	Text	Required Field	
Override Cycles	Text		
Paired End	Text Dropdown	Required Field	Presets • True • False

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Field Name	Field Type	Options	Additional Options and Dropdown Items
Read 1 Cycles	Numeric Dropdown	Required Field Custom Entries	Range = 1–251 Decimal places displayed = 0 Presets • 251* • 151 • 101 • 51 *The value of 251 is only supported for SP flow cell type. For all other flow cell types, the maximum value is 151.
Read 2 Cycles	Numeric Dropdown	Required Field Custom Entries	Range = 0–251 Decimal places displayed = 0 Presets • 251* • 151 • 101 • 51 *The value of 251 is only supported for SP flow cell type. For all other flow cell types, the maximum value is 151.
Reverse Complement Workflow	Toggle Switch	Required Field	Default • Yes
Run Mode (not displayed in user interface)	Text Dropdown	Read Only	Presets • SP • S1 • S2 • S4
Samplesheet Format	Text Dropdown	Required Field	Presets • V1 (default) • V2
Settings Header (not displayed in user interface)	Text	Read Only	

Field Name	Field Type	Options	Additional Options and Dropdown Items
UMI - Read 1 Length	Numeric		Range = 1
UMI - Read 1 Start From Cycle	Numeric		Range = 1
UMI - Read 2 Length	Numeric		Range = 1
UMI - Read 2 Start From Cycle	Numeric		Range = 1
Use Custom Index Read 1 Primer	Toggle Switch		Default • None Set
Use Custom Read 1 Primer	Toggle Switch		Default • None Set
Use Custom Read 2 Primer	Toggle Switch		Default • None Set
Use Custom Recipe	Toggle Switch	Required Field	Default • No
Validation Script	Multiline Text	Required Field Read Only	Default value provided in the *Validation Script* on page 411 section following the table. Do not remove this field as it is used by Validate Run Setup and Generate Sample Sheet automation script.
Workflow	Text	Read Only	Default • GenerateFASTQ
Workflow Type	Text Dropdown	Required Field	Presets • No Index • Single Index • Dual Index • Custom

Validation Script

```
step.::Run Mode:: = input.::Flowcell Type::; if (!step.::Experiment
Name::.matches(::[a-zA-Z0-9-_]+::)) { fail(::Experiment Name contains prohibited
characters. Allowed characters are: a-z, A-Z, 0-9, -, and _::) }; if
(step.::Workflow Type:: == ::No Index::) { if (step.::Index Read 1:: != 0 ||
step.::Index Read 2:: != 0) { fail(::Index Read 1 and Index Read 2 must be 0 if
No Index is selected.::) } } else if (step.::Workflow Type:: == ::Single
Index::) { if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) { fail
(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if Single
Index is selected.::) } } else { if (step.::Index Read 1:: == 0 || step.::Index
Read 2:: == 0) { fail(::Index Read 1 and Index Read 2 must be greater than 0 if
Dual Index is selected.::) } }; if (step.::Paired End::.toBoolean()) { if
(step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) { fail(::Read 1
Cycles and Read 2 Cycles must be greater than 0 if Paired End is True.::) } }
else { if (step.::Read 2 Cycles:: != 0) { fail(::Read 2 Cycles must be 0 if
Paired End is False.::) }; if (step.hasValue(::UMI - Read 2 Length::) ||
step.hasValue(::UMI - Read 2 Start From Cycle::)) { fail(::UMI - Read 2 Length
and UMI - Read 2 Start From Cycle cannot be defined if Paired End is False.::) }
}; if (input.::Flowcell Type:: != ::SP:: && step.::Read 1 Cycles:: > 151) { fail
(::Read 1 Cycles must not be larger than 151 if it is not SPrime Flowcell::) };
if (input.::Flowcell Type:: != ::SP:: && step.::Read 2 Cycles:: > 151) { fail
(::Read 2 Cycles must not be larger than 151 if it is not SPrime Flowcell::) };
if (step.hasValue(::UMI - Read 1 Length::) && !step.hasValue(::UMI - Read 1
Start From Cycle::)) { fail(::UMI - Read 1 Start From Cycle must be greater than
0 if UMI - Read 1 Length is greater than 0.::) }; if (!step.hasValue(::UMI -
Read 1 Length::) && step.hasValue(::UMI - Read 1 Start From Cycle::)) { fail
(::UMI - Read 1 Length must be greater than 0 if UMI - Read 1 Start From Cycle
is greater than 0.::) }; if (step.hasValue(::UMI - Read 2 Length::) &&
!step.hasValue(::UMI - Read 2 Start From Cycle::)) { fail(::UMI - Read 2 Start
From Cycle must be greater than 0 if UMI - Read 2 Length is greater than 0.::)
}; if (!step.hasValue(::UMI - Read 2 Length::) && step.hasValue(::UMI - Read 2
Start From Cycle::)) { fail(::UMI - Read 2 Length must be greater than 0 if UMI
- Read 2 Start From Cycle is greater than 0.::) }; if (!step.hasValue(::UMI -
Read 1 Length::) && step.hasValue(::UMI - Read 2 Length::)) { fail(::UMI - Read
1 Length must be greater than 0 if UMI - Read 2 Length is greater than 0.::) };
if (!step.hasValue(::UMI - Read 1 Start From Cycle::) && step.hasValue(::UMI -
Read 2 Start From Cycle::)) { fail(::UMI - Read 1 Start From Cycle must be
greater than 0 if UMI - Read 2 Start From Cycle is greater than 0.::) }; if
(step.hasValue(::Analysis Software Version::) && !step.::Analysis Software
Version::.matches(::^(\\d+)(\\.\\d+)*$::)) { fail(::Analysis Software Version
contains prohibited characters. Allowed characters are 0-9 and period. It shall
```


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```
start and end with numbers and separated by single period e.g. 3.8.4::) }; if
(step.hasValue(::Override Cycles::) && !step.::Override Cycles::.matches(::
[YNIU0-9;]+::)) { fail(::Override Cycles contains prohibited characters. Allowed
characters are: Y, N, I, U, 0-9 and ;. Example: N1Y150;I8;I7N1;Y141U10.::) };
step.::Settings Header:: = step.hasValue(::UMI - Read 1 Length::) ||
step.hasValue(::UMI - Read 2 Length::) ? ::[Settings]:: : ::::; nextStep =
::ADVANCE::
```

Global Fields

The following table lists the global custom fields that are configured to display on the Load to Flowcell (NovaSeq 6000 v3.7) step.

Table 133 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
Flowcell Type	Text Dropdown	Required Field	Presets • SP • S1 • S2 • S4
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User Interaction

Refer to Run Load to Flowcell (NovaSeq 6000 v3.7) step section in *NovaSeq 6000 Integration v3.5.1 Validation and Troubleshooting* on page 429.

Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7)

This final protocol contains one fully automated step, AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7), described in the following section.

This step is fully automated. Do not add samples to the Ice Bucket or start the step manually. The sequencing service does not update samples correctly when they have been manually started.

Step 1: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7)

Step input: Library tube from NovaSeq Standard or flow cell from NovaSeq Xp protocol

Step output: Result file/measurement

In this step, pooled samples are sequenced on the NovaSeq instrument and the run metrics are recorded in Clarity LIMS.

Set Next Steps Automation

By default, the Set Next Steps automation is disabled (set to Not Used) and the Sequencer API sets the next step for samples.

If necessary, override the default next step behavior used by the Sequencer API by performing the following steps:

1. Enable the automation and use it to set custom next step behavior and generate a log file. By default, the automation sets the next step to ADVANCE and the samples complete the protocol.
2. Configure the automation to trigger automatically on exit of the Record Details screen.
3. Disable or configure auto-complete behavior by setting the values of the run.autoComplete and autoCompleteOnlyAtSuccess properties. For details, refer to *Configuring Autocomplete Properties* on page 594.

Update Lane Number Automation

This automation triggers automatically upon entry to the Record Details screen.

Do not disable or modify this automation to ensure the Lane Number displays properly.

Master Step Fields

The following fields are configured on the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7) step in Clarity LIMS:

- Current Cycle
- Current Read
- Firmware Version
- Flow Cell Expiration Date
- Flow Cell ID
- Flow Cell Lot Number
- Flow Cell Mode
- Flow Cell Part Number
- Flow Cell Side
- Instrument Control Software Version
- Instrument ID
- Instrument Type

- Lane Counter
- Loading Workflow Type
- Output Folder
- RTA Version
- Run Completion Date
- Run ID
- Run Status
- Sequencing Log

Global Fields

The following fields are used to capture the run metrics in Clarity LIMS:

- % Aligned R1
- % Aligned R2
- % Bases >=Q30 R1
- % Bases >=Q30 R2
- % Error Rate R1
- % Error Rate R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- %PF R1
- %PF R2
- Cluster Density (K/mm^2) R1
- Cluster Density (K/mm^2) R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- Reads PF (M) R1
- Reads PF (M) R2
- Yield PF (Gb) R1
- Yield PF (Gb) R2

Note the following details:

- Values are aggregated across all lanes. Some values (eg, Yield PF (Gb) R1) are summed while others are averaged.
- The names listed previously are the default global custom field names installed with the NovaSeq Integration v3.5.1 configuration provided in the Illumina Preset Protocols (IPP) v2.5 or later.
- All global configuration fields are configured on the Container entity.
- All field names are configurable through the Custom Fields screen in the Global Fields tab.
- If field names are changed in Clarity LIMS, they must also be changed through the `run.metricUdfNames` properties file in the `application.yml` file for the Sequencer API. For more information, refer to the *application.yml Properties* section in *Components Installed* on page 421. All fields are configured to be visible in the Sample Details table on the Record Details screen for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7) step.

These global custom fields are configured on the Container entity, visible on each input, but they should not be confused with per-lane metrics.

All run metrics tracked in Clarity LIMS are overall metrics for the run. There are currently no per-lane metrics provided by NovaSeq Control Software.

Sample Sheet Generation

The sample sheet is generated on the step before the run. This step is Dilute and Denature (NovaSeq 6000 v3.7) in the NovaSeq Standard protocol or Load to Flowcell (NovaSeq 6000 v3.7) in the NovaSeq Xp protocol. This step places samples on the library tube or flow cell that are loaded in the NovaSeq 6000 instrument.

In the default configuration, the Validate Run Setup and Generate Sample Sheet automation generates one CSV format sample sheet file. The bcl2fastq v2.20 downstream analysis uses this file.

The sample sheet is uploaded to the NVCS via the `/Illumina/Sequencer/v2/sequencing-run/files` endpoint to the Sequencer API. The file endpoint allows for a file to be downloaded from the Clarity LIMS using OAuth (instead of Basic Authentication), which is required for the NovaSeq 6000 instrument.

The run recipe response sets the sample sheet URL to the link to download the file from this endpoint and sets the sampleSheetRequiresOAuth value to true.

Sample Sheet Content

The following master step fields control the sample sheet content. These fields display in the Step Details area of the Record Details screen.

- Experiment Name
- Read 1 Cycles
- Read 2 Cycles

- Workflow
- Reverse Complement Workflow
- Samplesheet Format

Depending on Samplesheet Format field value selection, a v1 or v2 sample sheet is generated. For detailed information on the following items, refer to the *Bcl2fastq2 Sample Sheet Generation* section of *Illumina Instrument Sample Sheets (NGS v5.17 & later)* on page 2542:

- Sample sheet generation script parameters and usage
- Sample sheet data and configuration options
- Enabling unique FASTQ file names per sequencing run

NovaSeq 6000 Run

The following steps outline the sequence of events that occurs when a flow cell is loaded onto the NovaSeq 6000 instrument.

User Authentication and Login

User authentication and login to the NovaSeq instrument is achieved via the getSequencerLoginURL endpoint (found at `/Illumina/Sequencer/v2/sequencing-run/login`). This endpoint returns the URL that the instrument uses to get an access token.

1. The Sequencer API service provides the NovaSeq Control Software (NVCS) with a URL for the Clarity LIMS login screen and the NVCS displays this screen to the user. Log in to authorize the NVCS to use your account automatically to access Clarity LIMS.
2. Enter credentials:
   - Username and password are passed to the Clarity LIMS server to make sure that the access is valid.
   - By default, the Sequencer API service tries to contact the Clarity LIMS at `http://localhost:9080/clarity/`. The installation script sets this URL, but it can be configured with the clarity.url property in the `application.yml` file. For more information, refer to the *application.yml Properties* section of *Components Installed* on page 421.
3. The user is redirected to the Sequencer login page at `/Illumina/Sequencer/v2/sequencer_login`.
   - If authentication is successful, the screen displays a success message. The Clarity LIMS server provides the OAuth token and username by returning them in redirect to `/Illumina/Sequencer/v2/sequencer_login/Success`. This redirect does not reload the page, but allows the NovaSeq to continue with run setup.
   - If authentication fails, the user is left on the login screen, and a warning message informs them of the failure. A background redirect to `/Illumina/Sequencer/v2/sequencer_login/Error` informs the NovaSeq 6000 of the failure.

Respond to Validation / Recipe Request Call from Instrument

1. The `sequencing-run/recipe/novaseq` endpoint consumes a POST request with a LibraryContainerId, FlowCellId, and reagents information.
   - LibraryContainerId, FlowCellId, and reagent information is provided by the NovaSeq 6000.
   - LibraryContainerId and FlowCellId can match either the library tube ID or flow cell ID of the corresponding containers in Clarity LIMS.
   - If there are no containers or multiple containers, an error is generated.
2. All populated containers whose name matches either the LibraryContainerId or FlowCellId are validated as follows.
   - All samples in the container must be queued for a sequencing run step.
   - All samples must be queued for only one sequencing run step.
   - All samples must be queued for the same sequencing run step.

   Configure the steps that are considered sequencing run steps in the novaseq.sequenceStepNames property in the `application.yml` file. The property is a list and each list entry must be the exact name of a sequencing step, not the name of the underlying master step/process type. For more information, refer to the *application.yml Properties* section of *Components Installed* on page 421.
3. After a single container has passed validation, the run setup information (run recipe request) is returned to the NVCS via the API. Then, the run recipe is generated.
   - The run recipe information is read from fields configured on the parent process/master step of the samples in the container. The workflow must include recipe definition in that step.
   - By default, the parent process/master step is the Dilute and Denature (NovaSeq 6000 v3.7) or Load to Flowcell (NovaSeq 6000 v3.7) step, depending on workflow.
   - The default field values match the names of the fields in the NovaSeq v3.7 workflow configuration included in IPP. The field names are configurable through the recipe.udfNames.* properties. Each of these properties corresponds to an entry in the recipe. The value of a property defines the name of the field that is used to populate that entry in the recipe.
   - It is assumed that this parent process/master step removes any illegal characters from the Experiment Name field.
   - The Clarity LIMS download link to the sample sheet created on the run setup step is used for the SampleSheet value in the recipe. The name of the output of the sample sheet on the setup step can be configured through the recipe.sampleSheet.outputName property (Sample Sheet by default) in the `application.yml` file. For more information, refer to the *application.yml Properties* section of *Components Installed* on page 421. If no matching output is found, or no file was attached to the output, then the value of the recipe.sampleSheet.notAvailableValue property (Not Available by default) is used in the recipe.

4. After the recipe is generated, it is returned as a *.json file in the response, in the form defined by the Swagger definition for the API.

Run Recipe Content

The sample sheet content and the fields configured on the Dilute and Denature (NovaSeq 6000 v3.7) and Load to Flowcell (NovaSeq 6000 v3.7) steps control the content of the run recipe.

The following example shows the run recipe content:

```
{
"run_name": "NVCS16027_SPrime_2x250",
"run_mode": "S4",
"workflow_type": "DualIndex",
"librarytube_id": "NV0025867-LIB",
"flowcell_id": "",
"sample_loading_type": "NovaSeqStandard",
"rehyb": false,
"paired_end": true,
"read1": 151,
"read2": 151,
"index_read1": 8,
"index_read2": 8,
"output_folder": "\\\\network_path\\run_data",
"samplesheet": "<path to sample sheet on LIMS server>",
"require_samplesheet_authentication": true,
"usecustomrecipe": false,
"customRecipe": null,
"use_basespace": false,
"basespace_mode": null,
"use_custom_read1_primer": false,
"use_custom_read2_primer": false,
"use_custom_index_read1_primer": false
}
```

Respond to Sequencing Started Call from Instrument

1. Matching container for the run (library tube or flow cell) is found, and contents are validated to be queued for the correct step.

   If no matching container is found, or if the samples in the container are incorrectly queued, an error occurs and the step is not started. This validation is also done during the run recipe request immediately prior, so this error case should not be encountered.

2. Reagent kits that are configured for the Sequencer API are checked against the configuration for the step to be run:
   - If a kit is configured for the API but does not exist in Clarity LIMS, it is created.
   - If a kit exists, but is not enabled on the step to be run, the step configuration is updated to enable it.
     - If a kit that is to be enabled is Archived, it is set to Active.
     - If a kit that is to be enabled is in Pending status, it is set to Active.
   - If any kits enabled on the step are not configured for the Sequencer API, a warning is logged. The API cannot complete the step without user intervention.
   - If any kits provided in the recipe request are not configured for the Sequencer API, they are ignored. A warning is logged during lot tracking.
3. The type of flow cell being used in the run determines the number of replicates for the step.
   - For Standard mode, this information is based on the number of lanes in the flow cell. This information is stored as part of the Sequencer API configuration.
   - For XP mode, there is always one replicate, because there is already one input tracked for each lane.
4. The automated sequencing step is started.
5. Reagent lots are attached to the step. The Flow Cell and Library Tube reagents in the request are ignored. Information from these reagents is tracked in Clarity LIMS as containers and custom fields. Any other reagents in the request are handled as follows.
   - If the reagent is not configured to be tracked, a warning is logged, and the reagent is ignored.
   - Existing reagent lots are looked up by kit name (determined by Sequencer API configuration), name (the serial number in the request), and lot number (the lot number in the request).
   - If one or more matching reagent lots exist in Clarity LIMS, the newest one is used.
   - If the expiry date does not match the expiry in the request, or if the status is not set to Active, the lot is updated to have the correct expiry date and an Active status.

   Otherwise a new lot is created with the following configuration:
   - Reagent Kit—Matching kit as determined from Sequencer API configuration and 2.
   - Name—Serial number in request
   - Lot Number—Lot number in request
   - Expiry—Expiry date in request
   - Status—Active

   If any reagents are expected to be in the request but are not, these reagents are logged. This action prevents the step from being completed without user intervention.

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6. Master step custom fields are updated with the run information parsed from the request. For more information, refer to *Respond to Record InterOps Metrics Call from Instrument* on page 420.
7. Any important events during this request are logged into the Sequencer API log file and in the Sequencing Log multiline global custom field on the step (assuming the step was started successfully). For more information, refer to *Respond to Record InterOps Metrics Call from Instrument* on page 420.

Respond to Record InterOps Metrics Call from Instrument

When the metrics endpoint receives a POST, it completes the following actions:

- Looks for a matching library tube or flow cell in Clarity LIMS.
- Find the in-progress sequencing step for that container.

When the step is found, the run metrics in the request are written to container global custom fields in Clarity LIMS.

Respond to Sequencing Completed Call from Instrument

If the sequencing run is successful, after the metrics are recorded, the API call for Sequencing Completed is received. Information is matched by Sequencing Container and the following occurs in Clarity LIMS:

1. The Current Cycle, Current Read, and Status master step custom fields are updated.
2. The multiline Sequencing Log master step field is updated.
3. A success message is recorded in the log file at the info level:

```
Sequencing run with Run ID {runId} of Step '{step.name}' ({step.limsid})
completed successfully.
```

4. The Clarity LIMS waits for any automations that are triggered automatically as it advances the step.
5. By default, the Clarity LIMS assigns the next steps on the outputs to move them on to the first configured next step. If there are no next steps, the protocol is completed.

Respond to Sequencing Errored Call from Instrument

If the sequencing run is unsuccessful, the API call for Sequencing Error is received. Information is matched by Sequencing Container and the following occurs in Clarity LIMS:

1. The Current Cycle, Current Read, and Status master step custom fields are updated.
2. The multiline Sequencing Log master step field is updated.
3. A failure message is recorded in the log file at the error level:

```
Step '${Step.name}' (${Step.limsid}) failed with Run ID ${runId}.
```

4. The step is left at the Record Details screen.

Respond to Sequencing RunEndedByUser Call from Sequencer

If you end a sequencing run, the API call for Sequencing RunEndedByUser is received. Information is matched by Sequencing Container and the following occurs in Clarity LIMS:

1. The Current Cycle, Current Read, and Status master fields are updated.
2. The multiline Sequencing Log master step field is updated.
3. The following message is recorded in the log file, at the warning level:

```
"Sequencing run with Run ID ${status.runInfo.runId} of Step
'${step.configuration.name}' (${step.limsid}) aborted by user
${status.runInfo.userName}."
```

4. The step is left at the Record Details screen.

Components Installed

The following sections describe the components (files, properties, reagent categories / label groups, reagent kits, and containers) that are installed by default as part of this integration.

Illumina NovaSeq 6000 Integration v3.5.1 is distributed as the `BaseSpaceLIMS-sequencer-api` RPM package. This RPM package must be installed on the Clarity LIMS server.

The `BaseSpaceLIMS-sequencer-api` RPM installs the following items:

- Sequencer API WAR file
- `application.yml` configuration file
- Two configuration scripts: `configure_sequencer_api_proxy.sh` and `configure_sequencer_api_application.sh`

The NovaSeq 6000 v3 configuration is delivered through Illumina Preset Protocols (IPP) v2.5, which requires installation of the NGS Extensions Package v5.22.0 or later.

- If the NGS Extensions Package is not already installed, or if a version earlier than v5.22.0 is installed, the latest version is installed by default with the NovaSeq 6000 integration. For more information, refer to *NovaSeq 6000 Integration v3.5.1 Release Notes* on page 453.
- If NGS Extensions Package v5.22.0 or later is already installed, the upgrade is not forced or required.

NovaSeq 6000 Integration RPM Components

The following table lists the components installed by the RPM package.

Files Installed	Location and Description
`Illumina#Sequencer#v2.war`	`/opt/gls/clarity/tomcat/current/webapps` War file for Sequencer API v2.4.1.

Files Installed	Location and Description
`configure_sequencer_api_application.sh`	`/opt/gls/clarity/config/` Script that configures the Sequencer API application through its external `application.yml` file.
`configure_sequencer_api_env.sh`	`/opt/gls/clarity/config/` Script that configures the Clarity LIMS Tomcat configuration to include Secret Util settings.
`configure_sequencer_api_proxy.sh`	`/opt/gls/clarity/config/` Script that configures the proxy to allow communication with the Sequencer API v2.4.1 application.
`sequencer-api.conf`	`/etc/httpd/clarity/` Proxy setting to communicate with the Clarity LIMS.

application.yml Properties

The `application.yml` file is at `opt/gls/clarity/extensions/sequencer-api/`.

- All properties are configured automatically during installation, but they can also be configured by editing the `application.yml` file and then restarting Tomcat.
- Properties in the file represent all custom field names, both for recipe and run information.
- For all custom fields, the names (values in this file) can be changed. However, the property names cannot be changed, removed, or added to. For example, Loading Workflow Type can be changed, but recipe.udfNames.sampleLoadingType cannot.

Table 134 application.yml Properties Installed

Property	Description
spring.profiles.active	Tells the application that it is running deployed in Tomcat. Default (do not change) • tomcat
clarity.url	Base URL that the Sequencer API service uses to contact the Clarity LIMS. The installation script (`configure_sequencer_api_application`) prompts for this URL.

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Property	Description
clarity.username	Username to be used when communicating with Clarity LIMS.
security.signing-key	Private key that is used when signing/validating OAuth tokens. Changing this key invalidates any issued tokens.
security.token-expiry	Specifies (in hours) the expiry period for login tokens issued by the Sequencer API. Default • 88
novaseq.sequenceStepNames	List of NovaSeq sequencing run step names for which the integration can find samples queued. • Names must be an exact match to the name of the step in Clarity LIMS (not the master step/process type). • Each line must be indented to the same point and start with a dash (-). Default - "AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7)" - "AUTOMATED - NovaSeqDx Run (NovaSeqDx v1.1)"

novaseq.flowcells	List of supported NovaSeq flow cell types.
	• Type names exactly match the values used by the NVCS run status requests.
	• To add more types, provide both type and lanes properties, indented to the same point and starting with a dash (-).
	Default
	`- type: "SP"`
	`lanes: 2`
	`- type: "S1"`
	`lanes: 2`
	`- type: "S2"`
	`lanes: 2`
	`- type: "S4"`
	`lanes: 4`

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Property	Description

novaseq.reagents	List of supported reagent kits to be tracked on sequencing steps.
	• **- lotName**—The name of the lot as it appears in the run started request.
	• - kitName—The name of the corresponding reagent kit in Clarity LIMS that the lot is tracked as.
	• The name can differ in the NVCS request versus the Clarity LIMS configuration.
	Default
	`- lotName: "Buffer"`
	`kitName: "Buffer Cartridge"`
	`- lotName: "Cluster"`
	`kitName: "Cluster Cartridge"`
	`- lotName: "SBS"`
	`kitName: "SBS Cartridge"`

recipe.udfNames	Configures the names of the fields used in the run recipe. For more information, refer to the *Run Recipe Contents* section of *NovaSeq 6000 Run* on page 416. • Each value must be enclosed in quotes (eg, recipe.udfNames.runMode "Run Mode"). • Value provided for sampleLoadingType must match the value provided in the run configuration (eg, recipe.udfNames.sampleLoadingType "Loading Workflow Type").
recipe.sampleSheet.outputName recipe.sampleSheet.notAvailableValue	Configures the name of the sample sheet file placeholder on the step where run recipe information is populated, and the property value to use when the file is not found.

Property	Description
run.stepUdfNames	Configures the master step field names to be used when recording run results. For more information, refer to the *Master Step Fields* section of *Step 1: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7)* in *Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7)* on page 412. • Each value must be enclosed in quotes (eg, run.stepUdfNames.runID "Run ID") • Value provided for sampleLoadingType must match the value provided in the run configuration (eg, run.stepUdfNames.sampleLoadingType "Loading Workflow Type"). It is expected that the run step field and the field on the input derived sample share a name.
run.metricUdfNames	Configures the result file custom fields/measurement global field names to be used when recording run results. These fields capture the run data. For more information, refer to the *Global Fields* section of *Step 1: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7)* in *Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7)* on page 412.
run.autoComplete	Determines whether the sequencing run step must be completed automatically after parsing the run metrics on a run along with run.autoCompleteOnlyAtSuccess. For more information, refer to *Configuring autoComplete Properties* on page 427. Default • true

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Property	Description
run.autoCompleteOnlyAtSuccess	Determines which of the following options that the sequencing run step must be: • Autocompleted when the run status is RunCompletedSuccessfully (if autoCompleteOnlyAtSuccess is true) • Autocompleted regardless of the run status (if autoCompleteOnlyAtSuccess is false). For more information, refer to *Configuring autoComplete Properties* on page 427. Default • true

Reagent Categories/Label Groups

- TruSeq HT Adapters v2 (D7-D5)

Reagent Kits

- Buffer Cartridge
- Cluster Cartridge
- DPX1
- DPX2
- DPX3
- NaOH
- Resuspension Buffer (RSB)
- SBS Cartridge
- Tris HCl

Container Types

- Library Tube
- SP
- S1
- S2
- S4

This integration supports the following items:

- Library tube with barcode provided in the format [A-Z]{2}[0-9]{7}-[A-Z]{3} (eg, AB1234567-XYZ)
- SP, S1, S2, or S4 flow cell with barcode provided in one of the following formats:
  - SP and S1 flow cell: [A-Z0-9]{5}DR[A-Z0-9]{2}
  - S2 flow cell: [A-Z0-9]{5}DM[A-Z0-9]{2}
  - S4 flow cell: [A-Z0-9]{5}DS[A-Z0-9]{2}
  - Example: H1991DMXX

NVCS Configuration

For details on configuring NVCS for integration with Clarity LIMS, contact the Clarity LIMS Support team.

Routing Script Requirements

The requirements for the routing script functionality are as follows.

1. On the steps that use the routing script (Define Run Format (NovaSeq 6000 v3.7) and Dilute and Denature (NovaSeq 6000 v3.7), the Next Step for all samples must be set to Remove from workflow. A script sets this value. The value must not change in the Assign Next Steps screen.
2. In the Next Steps section of the protocol configuration screen, the method of assigning the next step must be set to Automation for the last step of the protocol.

Configuring autoComplete Properties

As of NovaSeq Integration v3.5.1, the `application.yml` configuration file contains two additional properties:

- run.autoComplete
- run.autoCompleteOnlyAtSuccess

These properties determine the conditions under which the last step of the NovaSeq 6000 workflow (AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7)) is automatically completed when the run status is "RunCompletedSuccessfully" (default behavior) or regardless of the run status.

By default, both properties are set to true and the step only completes when the run is successful. If the run fails or is aborted, manually complete the step in Clarity LIMS.

For the step to autoComplete regardless of the result of the sequencing run, change the autoCompleteOnlyAtSuccess property value to false.

Enabling/Disabling autoComplete Properties

1. Open the `application.yml` file at `/opt/gls/clarity/extensions/sequencer-api/application.yml`:

```
vi /opt/gls/clarity/extensions/sequencer-api/application.yml
```

2. Edit the value of the autoCompleteOnlyAtSuccessproperty as required.

3. Save the file.

The following table shows how the combined value of the run.autoComplete and run.autoCompleteOnlyAtSuccess properties affects the autoComplete behavior of the sequencing step.

Table 135 run.autoComplete and run.autoCompleteOnlyAtSuccess Value Matrix

run.autoComplete Property Value	run.autoCompleteOnlyAtSuccess Property Value	Outcome
true	true	• Step automatically completes only if sequencing run is successful. • If run is not successful, step does not automatically complete, and the run details are recorded in Clarity LIMS.
true	false	• Step automatically completes, regardless of the run status.
false	true	• Step does not automatically complete. • Run details are recorded in Clarity LIMS.
false	false	

Rules and Constraints

- The workflow configuration contains several validation checks. To make sure that the calculations work properly, it is important that you do not disable any of this validation logic. The validation checks determine the following information:
  - Which samples, and how many, can enter each step together.
  - Which samples, and how many, can be pooled together.
- The library tube ID must be unique. There must not be multiple library tube containers in the system with the same name.
- Reagent labels (indexes) must be unique.
- Only controls are permitted as unindexed samples; all other unindexed samples and pools are not permitted.
- For sample sheet generation constraints, refer to the *Bcl2fastq2 Sample Sheet Generation* section in *Illumina Instrument Sample Sheets (NGS v5.17 & later)* on page 2542.

- Do not manually start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7) step. This step is a fully automated and the sequencing service does not update samples correctly if they have been manually started.
- For the automated run to start successfully, Validate Run Setup and Generate Sample Sheet must be selected.

NovaSeq 6000 Integration v3.5.1 Validation and Troubleshooting

This section explains how to validate the installation of the Illumina NovaSeq 6000 Integration Package v3.5.1.

The validation process involves the following items:

- Running samples through the Library Prep Validation workflow.
  - The workflow contains a single-step protocol that models the library prep required to produce normalized libraries. At the end of the step, the normalized libraries advance to the workflow selected.
- Running normalized libraries through the NovaSeq 6000 v3.7 workflow validates the following items:
  - Successful routing of samples from the Run Format (NovaSeq 6000 v3.7) step to the NovaSeq Standard (NovaSeq 6000 v3.7) or NovaSeq Xp (NovaSeq 6000 v3.7) step.
  - Automated generation of v1 sample sheet for use with bcl2fastq2 v2.20 analysis software or v2 sample sheet. This file automatically uploads to the sequencing system via the Sequencer API.
  - Automatic validation of run setup information. Information is uploaded to the NovaSeq Control Software (NVCS) via the Sequencer API and is used to create the run recipe and initiate the run.
  - Automated tracking of the NovaSeq sequencing run and parsing of run statistics into Clarity LIMS, via the Sequencer API.

The validation steps assume that the Illumina NovaSeq 6000 Integration Package v3.5.1 is installed and you have imported the default Clarity LIMS configuration.

Activate Workflow, Create Project, Add and Assign Samples

The following steps set up Clarity LIMS in preparation for running samples through the Library Prep Validation and NovaSeq 6000 v3.7 workflows.

1. On the Configuration tab, under Workflows, activate both the Library Prep Validation and NovaSeq 6000 v3.7 workflows.
   - Library Prep Validation v2.3.0 is available with Illumina Preset Protocols (IPP) v2.5.
2. On the Projects and Samples screen, create a project and add samples to it.
3. Assign the samples to the Library Prep Validation workflow.

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Library Prep Protocol: Library Prep Validation v2.3.0

This single-step protocol models the library prep required to produce normalized libraries that are ready for the NovaSeq 6000 v3.7 workflow.

Run Library Prep Validation v2.3.0

1. In Lab View, locate the Library Prep Validation v2.3.0 protocol.
   Samples are queued for the Library Prep Validation v2.3.0 step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Placement screen, complete the following actions:
   a. In the Placed Samples area on the right, in the Container field, scan or enter the barcode of the destination container.
   b. Select the samples from the Samples to be Placed area on the left side, and drag them to the container wells on the right side.
   c. Select Add Labels.
5. On the Add Labels screen, the Labels to Add list is populated with labels from the TruSeq HT Adapters v2 (D7-D5) label group. The group is the only label group configured in the integration and is used by default in this step.
   a. Select labels on the left side, and drag them to the container on the right side to assign them to placed samples.
   b. Select Record Details.
6. On the Record Details screen, complete the following actions:
   a. In the Sample Details table, enter values for Normalized Molarity (nM). Type values individually or copy and paste to enter multiple values from a spreadsheet.
   b. The Sequencing Instrument column indicates the instrument to be used for the sequencing run. Select NovaSeq 3.0.
   c. Populate the fields in this column using one of the following methods:
      - To populate fields individually for each sample, select from the drop-down list in each row.
      - To populate all fields at one time, copy and paste from a spreadsheet.

   Alternatively, select inside the table and press Ctrl + A to select all rows. At the top of the table, select Sequencing Instrument and then select NovaSeq 3.0 from the adjacent drop-down list. Select Apply.

   If working with many samples, copy and paste to insert multiple values from a spreadsheet as follows.

   a. Select Copy Samples to copy the sample information from Clarity LIMS to the Clipboard.
   b. Open a blank Excel document and paste the contents of the clipboard into the spreadsheet.

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c. In the spreadsheet, enter the Normalized Molarity and Sequencing Instrument values. Select the entire spreadsheet including the headers (Ctrl + A), and copy (Ctrl + C) the information to the Clipboard.

d. Within Clarity LIMS, select the Paste Samples button. Paste the contents of the clipboard (Ctrl + V) into the Update Table window and then select Update to paste the contents into the Sample Details table.

7. Select Next Steps.
   Triggers the Next Steps automation, which sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step.
8. On the Assign Next Steps screen in the Samples table, the Next Step value for all samples is prepopulated with Remove from workflow.

   Do not change this value. If Next Step is not set to Remove from workflow, the routing script does not route samples correctly.

9. Select Finish Step.
   On exit from the step, the Routing Script automation is triggered. This automation assigns samples to the first step of the NovaSeq 6000 v3.7 workflow, Define Run Format (NovaSeq 6000 v3.7). This step is the only one in Protocol 1: Run Format (NovaSeq 6000 v3.7).

Protocol 1: Run Format (NovaSeq 6000 v3.7)

This protocol includes a single step, Define Run Format (NovaSeq 6000 v3.7). The step allows for the assignment of per sample values for Loading Workflow Type, Normalized Molarity, Flowcell Type, and Final Loading Concentration (pM). At the end of the step, samples are routed to the NovaSeq Standard or NovaSeq Xp protocol, according to the selected Loading Workflow Type.

Step 1: Run Define Run Format (NovaSeq 6000 v3.7)

1. In Lab View, locate the Run Format (NovaSeq 6000 v3.7) protocol. The samples are queued for the Define Run Format (NovaSeq 6000 v3.7) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Record Details screen in the Sample Details table, populate the following fields (values can vary across samples):
   - Loading Workflow Type—Select NovaSeq Standard or NovaSeq Xp from the drop-down list.
   - Flowcell Type—Select SP, S1, S2, or S4.
   - Final Loading Concentration (pM)—Select from the two preset options: 225 (for PCR-free workflows), 400 (for Nano workflows), or enter a different value.

- Normalized Molarity (nM)—These values are copied over from the previous step. If this column is not populated during library prep, enter the values here.

5. Select Next Steps, which triggers the Next Steps automation and the following actions:
   - Sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step.
   - Calculates the Minimum Molarity.
   - Checks Normalized Molarity value. For samples with no Normalized Molarity value (an empty value not including 0), generates an error message notifying that the field cannot be empty.
   - Compares each sample Normalized Molarity value with the Minimum Molarity value.
6. On the Assign Next Steps screen:
   - In the Sample Details table, the Next Step for all samples is prepopulated with Remove from workflow, regardless of the Loading Workflow Type.

     Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not routed samples correctly.

   - For samples whose Normalized Molarity value is lower than the Minimum Molarity value, the Loading Workflow Type is set to Remove from workflow. Also, a message is recorded in the Warning field for the sample.
     At this point, there are two options:
     - Return to the Record Details screen and adjust the Normalized Molarity value so that it equals or exceeds the Minimum Molarity value. The Loading Workflow Type must be set to NovaSeq Standard or NovaSeq Xp, as applicable.
     - Complete the protocol without correcting the Normalized Molarity value. In this case, the samples in question are removed from the Clarity LIMS workflow.
7. Select Finish Step. The Routing Script automation is triggered:
   - Samples whose Loading Workflow Type is set to Remove from workflow are removed from the Clarity LIMS workflow. (Occurs when the Normalized Molarity value is lower than the Minimum Molarity.)
   - Samples whose Loading Workflow Type is set to NovaSeq Standard are routed to the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.7) step. This step is the first one in the NovaSeq Standard protocol.
   - Samples whose Loading Workflow Type is set to NovaSeq Xp are routed to the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.7) step. This step is the first one in the NovaSeq Xp protocol.

Protocol 2: NovaSeq Standard (NovaSeq 6000 v3.7)

In this protocol, samples are pooled and added to the library tube in preparation for the NovaSeq run.

Step 1: Run Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.7)

1. In Lab View, locate the NovaSeq XP (NovaSeq 6000 v3.7) protocol. Samples for the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.7) step are queued.
2. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Pooling screen:
   a. Create a pool by dragging samples into the Pool Creator.

      Only create one pool.

   b. Enter a name for the pool or accept the default name (Pool #1).
   c. Select Record Details.
      After exiting from the Pooling screen, the Validate Inputs Flowcell Type and Single Pool automation is triggered. The automation verifies the following items:
      - All samples in the pool have been assigned the same Flowcell Type.
      - Only one pool has been created.
5. On the Record Details screen in the Step Details area, complete the two required fields and one optional field, as needed:
   - Number of Flowcells to Sequence—Used in volume calculations, to make sure that the volumes are sufficient for the number of times the pool is sequenced.
   - Minimum Per Sample Volume (µl)—Used to calculate how much of each sample is included in the pool. The field is prepopulated with the configured default value 5 µl, but can be edited.
   - % PhiX (2.5 nM) Spike-In—Used to calculate the volume of PhiX v3 control included in the pool for the given percentage of spike-in. The field is optional and can be edited.

     Assuming the default Minimum Per Sample Volume (µl) value of 5, for a given batch:
     - If the smallest Per Sample Volume (µl) value is < 5, Clarity LIMS automatically assigns a value of 5 to the Adjusted Per Sample Volume (µl) field.
     - Clarity LIMS changes the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.
6. In the Sample Details table, select the pool icon to view details on the pool composition.
7. Select Calculate Volumes to trigger the Calculate Volumes automation. This automation performs calculations based on the selected Flowcell Type.
   - Calculates Bulk Pool Volume (µl) value.
   - Sets the value of the Bulk Pool Volume (µl) field.

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- Calculates the PhiX Volume (µl) value when % PhiX (2.5 nM) Spike-In has value.
- Calculates the Per Sample Volume (µl) value to be added to the pool.
- Calculates the Total Sample Volume (µl) value.
- If the Total Sample Volume is less than the Bulk Pool Volume, selects the RSB Volume (µl) value.
- Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
- Populates the Volume of Pool to Denature (µl), NaOH Volume (µl) and Tris-HCl Volume (µl) columns of the Sample Details table. The script sets the values, and is not editable.
- The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains volume information about the pool and the individual samples that it contains. Select on the file to download it and then open it in Excel.

8. Select Next Steps to trigger the Set Next Step automation. This automation sets the next step for samples to ADVANCE and advances them to the next step in the protocol, Dilute, and Denature (NovaSeq 6000 v3.7).
   On the Assign Next Steps screen, the next step for samples is set to the next step in the workflow: Dilute and Denature (NovaSeq 6000 v3.7).
9. Select Finish Step.
   At the end of this step, the pool of samples automatically advances to the Dilute and Denature (NovaSeq 6000 v3.7) step.

Step 2: Run Dilute and Denature (NovaSeq 6000 v3.7)

1. In Lab View, locate the NovaSeq Standard (NovaSeq 6000 v3.7) protocol. The pool of samples queued for the Dilute and Denature (NovaSeq 6000 v3.7) step are listed.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work. The Validate Single Input automation is triggered. This automation verifies that only one container is input for the step.
4. On the Placement screen:
   a. Drag the pool into the library tube in the Placed Samples area.
   b. Scan or type the barcode of the library tube into the Library Tube field.
   c. Select Record Details.
   After exiting the Placement screen, the Validate Library Tube Barcode automation makes sure that the library tube barcode conforms to the barcode mask [A-Z]{2}[0-9]{7}-[A-Z]{3}. If it does not, an error message displays. The automation also copies the Flowcell Type and Loading Workflow Type values from step inputs to outputs.
5. On the Record Details screen, the Reagent Lot Tracking section lets you tracks the NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. Add and activate lots for these reagents.

To add and activate reagent lots:

a. In a new browser tab, open an additional instance of Clarity LIMS.
b. Navigate to the Consumables configuration screen.
c. Select the NaOH reagent kit and select New Lot.
d. Enter the lot details.
e. Set the Status of Reagent Lot toggle switch to Active.
f. Select Save.
g. Repeat this process to add and activate Resuspension Buffer and Tris-HCl reagent lots.
h. Return to your original working browser tab and refresh the page.

6. In the Reagent Lot Tracking section, select from the active lots displayed in each drop-down list.
7. The fields displayed on the Record Details screen are used to set up the run and generate the sample sheet. Some of these fields are autopopulated and some must be completed manually.

Table 136 Fields Displayed on Record Details Screen of Denature and Dilute (NovaSeq 6000 v3.7)

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.
BaseSpace Sequence Hub Configuration	Presets • Not Used • Run Monitoring Only • Run Monitoring and Storage
Workflow	Preset • GenerateFASTQ
Workflow Type	Presets • No Index • Single Index • Dual Index • Custom
Index Read 1	Presets • 0 • 6 • 8 • type a value between 0 and 20

Field	Value
Index Read 2	Presets • 0 • 6 • 8 • type a value between 0 and 20
Paired End	Presets • True • False
Read 1 Cycles	Presets • 251* • 151 • 101 • 51 • type a value between 1 and 251 *The value of 251 is only supported for SP flow cell type. For all other flow cell types, the maximum value is 151.
Read 2 Cycles	Presets • 251* • 151 • 101 • 51 • type a value between 0 and 251 *The value of 251 is only supported for SP flow cell type. For all other flow cell types, the maximum value is 151.
Use Custom Read 1 Primer	Select if applicable.
Use Custom Read 2 Primer	Select if applicable.
Use Custom Index Read 1 Primer	Select if applicable.
Reverse Complement Workflow	Select Yes (by default) for v1.5 reagents in NovaSeq Control Software v1.7 and onwards. Select No for v1 reagents.
Use Custom Recipe	Select if applicable.
Custom Recipe Path	If the Use Custom Recipe option is selected, enter the path to the custom recipe file to be used.
UMI - Read 1 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 1. Leave blank if not applicable.

Field	Value
UMI - Read 2 Length	Enter the length of the UMI in Read 2. Leave blank if not applicable.
UMI - Read 1 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.
UMI - Read 2 Start From Cycle	Enter the cycle number that Read 2 starts from. Leave blank if not applicable.
Output Folder	Enter network path for sequencing run folder (eg, `\\networkshare\run_data`)
Samplesheet Format	Presets • V1 • V2
Analysis Software Version	Enter the software version for BCL Convert Analysis for v2 sample sheet. Leave blank if not applicable.
Override Cycles	Enter the Override Cycles value for v2 sample sheet. Leave blank if not applicable.

8. On the Record Details screen, select Validate Run Setup and Generate Sample Sheet. This selection triggers the automation script, which performs the following actions:
   - Validates the parameters entered on the Record Details screen.
   - Generates the sample sheet and attaches it to the placeholder in the Files area of the Record Details screen.

     The v2 sample sheet generated does not include Cloud and Analysis sections. Contact Illumina Support for information on generating v2 sample sheets with those sections.

   - Select Next Steps.
9. On the Assign Next Steps screen, the Next Step for samples is prepopulated with Remove from workflow. The Routing Script automation expects this value, and requires the value to advance samples to the next step.

   Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.

10. Select Finish Step.
    After exiting the step, the following actions occur:
    - The Routing Script automation is triggered and samples are routed to AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7).
    - In Lab View, the pool of samples is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7) step.

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At this point in the workflow, the user interaction ends. Proceed to AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7).

Do not add samples to the Ice Bucket or start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7) step. The integration adds samples automatically.

Protocol 3: NovaSeq Xp (NovaSeq 6000 v3.7)

In this protocol, samples are pooled and added to lanes on the NovaSeq flow cell. The option selected in the Define Run Format (NovaSeq 6000 v3.7) step determines the flow cell type.

Step 1: Run Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.7)

1. In Lab View, locate the NovaSeq Xp (NovaSeq 6000 v3.7) protocol. The samples are queued for the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.7) step.
2. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Pooling screen, create and name a pool as follows.
   a. Create a pool by dragging samples into the Pool Creator.

      Create only one pool.

   b. Enter a name for the pool or accept the default name (Pool #1).
   c. Select Record Details.
5. On exiting the Pooling screen, the Validate Inputs Flowcell Type and Single Pool automation is triggered. The automation verifies the following items:
   - All samples in the pool are assigned the same Flowcell Type.
   - Only one pool was created.
6. On the Record Details screen in the Step Details area, complete the two required fields and one optional field, as needed:
   - Number of Lanes to Sequence—Used in volume calculations to make sure that volumes are sufficient for the number of times the pool is sequenced.
   - Minimum Per Sample Volume (µl)—Used to calculate how much of each sample is included in the pool. The field is prepopulated with the configured default value 5 µl, but can be edited.
   - % PhiX (0.25nM) Spike-In—Used to calculate the volume of PhiX v3 control to be included in the pool for the given percentage of spike-in. The field is optional and can be edited.

Assuming the default Minimum Per Sample Volume (µl) value of 5, for a given batch:

- If the smallest Per Sample Volume (µl) value is < 5, Clarity LIMS automatically assigns a value of 5 to the samples Adjusted Per Sample Volume (µl) field.
- Clarity LIMS then adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch. This adjustment is based on the ratio used to increase the lowest value to 5.

7. In the Sample Details table, select the pool icon to view details on the pool composition.
8. Select Calculate Volumes. This selection triggers the Calculate Volumes automation. This automation performs volume calculations based on the selected Flowcell Type, as follows.
   - Calculates Bulk Pool Volume (µl) value.
   - Calculates the Per Sample Volume (µl) value to be added to the pool.
   - Calculates PhiX Volume (µl) value when % PhiX (0.25nM) Spike-In has value.
   - Calculates the Total Sample Volume (µl) value.
   - If the Total Sample Volume is less than the Bulk Pool Volume, calculate the RSB Volume (µl) value.
   - Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
   - Generates the Calculation File (CSV) and attaches it to the step. This file contains volume information about the samples and RSB buffer to add to the pool. Select the file to download it and then open it in Excel.
9. Select Next Steps to trigger the Set Next Step automation. This automation performs the following actions:
   - Copies the Flowcell Type values from the step inputs to the step outputs.
   - Sets the next step for samples to ADVANCE, advancing them to the Dilute, Denature, and ExAmp (NovaSeq 6000 v3.7) step in the protocol.

   On the Assign Next Steps screen, the next step for samples is set to the Dilute, Denature, and ExAmp (NovaSeq 6000 v3.7) step in the workflow.
10. Select Finish Step.
    At the end of this step, the pool of samples automatically advances to the Dilute, Denature, and ExAmp (NovaSeq 6000 v3.7) step.

Step 2: Run Dilute, Denature and ExAmp (NovaSeq 6000 v3.7)

1. In Lab View, locate the NovaSeq Xp (NovaSeq 6000 v3.7) protocol. The pool of samples queued for the Dilute, Denature, and ExAmp (NovaSeq 6000 v3.7) step displays.
2. Add the pool to the Ice Bucket and select View Ice Bucket.

3. [Optional] On the Ice Bucket screen, set the number of derivatives to create (placed into the flow cell lanes) and select Begin Work.
4. On entry to the Record Details screen, the Calculate Volumes automation is triggered.
   This automation sets the following values based on the selected Flowcell Type:
   - BP Aliquot Volume (µl)
   - NaOH Volume (µl)
   - Tris-HCl Volume (µl)
   - DPX1 Volume (µl)
   - DPX2 Volume (µl)
   - DPX3 Volume (µl)
   - Mastermix per Lane (µl)
   - The automation also populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
   - The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains information about the DPX Mastermix volume and the volume of Mastermix, NaOH, and Tris-HCl to add per working pool (see the next step).
5. On the Record Details screen, the Reagent Lot Tracking section tracks the DPX1, DPX2, DPX3, NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. These reagents must be added.
   To add and activate reagent lots:
   a. In a new browser tab, open an additional instance of Clarity LIMS.
   b. Select one of the reagent kits previously listed and select New Lot.
   c. Enter the lot details.
   d. Set the Status of Reagent Lot toggle switch to Active.
   e. Select Save.
   f. Repeat this process to add and activate the remaining reagent lots.
   g. Return to your original working browser tab and refresh the page.
6. On the Record Details screen:
   - In the Reagent Lot Tracking section, select from the active lots displayed in each drop-down list.
   - In the Step Details area, the DPX1, DPX2, and DPX3 reagent volume values are prepopulated. The script sets the values and they are not editable.
   - In the Sample Details table, select the pool icon to view details on the working pool composition.
     - The BP Aliquot, Mastermix per lane, NaOH, and Tris-HCl volume values for each working pool are populated. The script sets the values and they are not editable.

- The Flowcell Type and Loading Workflow Type columns are populated.
- The working pool number is appended to the bulk pool name, to identify which working pools are derived from the same bulk pool.

7. In the Files area, select the Calculation File (CSV) to open it and view details on the DPX Mastermix volume and the volume of Mastermix, NaOH, and Tris-HCl to add per working pool.
8. Select Next Steps. On the Assign Next Steps screen, the next step is already set to Load to Flowcell (NovaSeq 6000 v3.7).
9. Select Finish Step.

Step 3: Run Load to Flowcell (NovaSeq 6000 v3.7)

1. On the Ice Bucket screen:
   - In the Container Options panel, select the appropriate flow cell type from the Destination Container drop-down list.
   - Select Begin Work.
2. The Validate Inputs and Selected Container automation checks the following:
   - The Flowcell Type field is set to a valid value (SP, S1, S2, or S4) and that each input has the same value for the field.
   - The container type selected matches the value in the Flowcell Type field.
   - The number of outputs matches the number of lanes on the selected flow cell type. If validation fails, an error message reports the number of working pools does not match the number of lanes available on the flow cell.
3. On the Placement screen:
   - Drag one or more pools from the left of the screen over into the Placed Samples area on the right.
   - Scan or type the barcode of the flow cell into the Flowcell field.
   - Select Record Details.

   After exiting the Placement screen, the Validate Flowcell Barcode automation makes sure that the container barcode conforms to the barcode mask for the selected Flowcell Type (as listed in the following table). This automation also copies the Flowcell Type and Loading Workflow Type field values from step inputs to outputs.

Flowcell Type	Barcode Mask
SP	[A-Z0-9]{5}DR[A-Z0-9]{2}
S1	[A-Z0-9]{5}DR[A-Z0-9]{2}
S2	[A-Z0-9]{5}DM[A-Z0-9]{2}
S4	[A-Z0-9]{5}DS[A-Z0-9]{2}

4. The fields displayed on the Record Details screen are used to set up the run and generate the sample sheet.
   Some of these fields are autopopulated and some must be entered manually. For details, refer to the following table:

   Table 137 Fields Displayed on Record Details Screen of Load to Flowcell (NovaSeq 6000 v3.7) Step

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.
BaseSpace Sequence Hub Configuration	Presets • Not Used • Run Monitoring Only • Run Monitoring and Storage
Workflow	Preset • GenerateFASTQ
Workflow Type	Presets • No Index • Single Index • Dual Index • Custom
Index Read 1	Presets • 0 • 6 • 8 • type a value between 0 and 20
Index Read 2	Presets • 0 • 6 • 8 • type a value between 0 and 20
Paired End	Presets • True • False

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Field	Value
Read 1 Cycles	Presets • 251* • 151 • 101 • 51 • type a value between 1 and 251 *The value of 251 is only supported for SP flow cell type. For all other flow cell types, the maximum value is 151.
Read 2 Cycles	Presets • 251* • 151 • 101 • 51 • type a value between 0 and 251 *The value of 251 is only supported for SP flow cell type. For all other flow cell types, the maximum value is 151.
Use Custom Read 1 Primer	Select if applicable.
Use Custom Read 2 Primer	Select if applicable.
Use Custom Index Read 1 Primer	Select if applicable.
Reverse Complement Workflow	Select Yes (by default) for v1.5 reagents in NovaSeq Control Software v1.7 and onwards. Select No for v1 reagents.
Use Custom Recipe	Select if applicable.
Custom Recipe Path	If you selected the Use Custom Recipe option, enter the path to the custom recipe file to be used.
UMI - Read 1 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 1. Leave blank if not applicable.
UMI - Read 2 Length	Enter the length of the UMI in Read 2. Leave blank if not applicable.
UMI - Read 1 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.
UMI - Read 2 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.

Field	Value
Output Folder	Enter network path for sequencing run folder (eg, `\\networkshare\run_data`)
Library Tube Barcode	Scan the library tube barcode.
Samplesheet Format	Presets • V1 • V2
Analysis Software Version	Enter the software version for BCL Convert Analysis for v2 sample sheet. Leave blank if not applicable.
Override Cycles	Enter the Override Cycles value for v2 sample sheet. Leave blank if not applicable.

5. Select Validate Run Setup and Generate Sample Sheet to trigger the automation script, which does the following:
   - Validates the parameters entered on the Record Details screen.
   - Generates the sample sheet and attaches it to the placeholder in the Files area on the Record Details screen.

     The v2 sample sheet generated does not include Cloud and Analysis sections. Contact Illumina Support for information on generating v2 sample sheets with these sections.
6. Select Next Steps.
   On the Assign Next Steps screen, the Next Step field for samples is prepopulated with Mark protocol as complete.
7. Select Finish Step.
   At this point in the workflow, the user interaction ends. The flow cell is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7) step.

Proceed to AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7).

Do not add samples to the Ice Bucket or start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7) step. The integration adds samples automatically.

Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7)

This protocol contains a single fully automated step - AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7).

The integration starts the step automatically and data from the run is parsed back into Clarity LIMS. User interaction is not required, but the various stages can be reviewed in Clarity LIMS under Review Run Data.

Automations

The NovaSeq Run (NovaSeq 6000 v3.7) step contains two automations:

- Set Next Steps—This automation is disabled (Set to Not Used). By default, the Sequencer API is used to set the next step for output samples.
  The Illumina Preset Protocols (IPP) include a Set Next Steps automation configured on the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7) step. You can use this automation to override the default next step behavior. For details, refer to *NovaSeq 6000 Integration v3.5.1 Configuration and User Interaction* on page 369for details.
- Update Lane Number—This automation is triggered automatically upon entry to the Record Details screen.
  Do not disable or modify this automation to make sure that the Lane Number displays properly.

Review Run Data

Read summary metrics are recorded for the library pool. After the run is complete, open the step and review these metrics on the Next Steps screen in the Step Data section and the Sample Details table.

Step Data Section

The following values populate in the master step fields:

- Run ID
- Run Status
- Output Folder
- Current Cycle
- Current Read
- Loading Workflow Type
- Flow Cell ID
- Flow Cell Side
- Flow Cell Mode
- Flow Cell Part Number
- Flow Cell Lot Number
- Flow Cell Expiration Date
- Instrument ID
- Instrument Type
- Instrument Control Software Version
- Firmware Version

- RTA Version
- Sequencing Log

Sample Details Table

Summary metrics populate the global fields listed. Values are aggregated across all lanes. Some values (for example Yield PF (Gb) R1) are summed, while others are averaged.

- Yield PF (Gb) R1
- Yield PF (Gb) R2
- Reads PF (M) R1
- Reads PF (M) R2
- Cluster Density (K/mm^2) R1
- Cluster Density (K/mm^2) R2
- %PF R1
- %PF R2
- % Bases >=Q30 R1
- % Bases >=Q30 R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- % Aligned R1
- % Aligned R2
- % Error Rate R1
- % Error Rate R2

How the Integration Works

The following steps summarize how the Sequencer API integration works. For details on sample sheet generation and the sequence of events involved in the NovaSeq run, refer to *NovaSeq 6000 Integration v3.5.1 Configuration and User Interaction* on page 369.

1. When setting up a run on the NovaSeq 6000 instrument, NVCS sends a request for the run recipe.
    a. The Sequencer API validates that samples and containers are correctly queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7) step.

b. Clarity LIMS sends a JSON response to NVCS. The response includes the run recipe information and a link to download the sample sheet (used with analysis software such as bcl2fastq2).

2. When the run starts, NVCS sends a RunStarted run status request. The Sequencer API then does the following:
   a. Validates reagent kit information in the RunStarted request:
      - Verifies that the reagent kit exists. If the reagent kit does not exist, the Sequencer API creates and enables it on the master step.
      - Verifies that the reagent kit is activated. If the reagent kit is not active, the Sequencer API activates it.
   b. Initiates the step for the queued samples. The step produces one output file placeholder per lane of the flow cell in use, based on the flow cell type.
   c. Records all relevant information from the RunStarted request on the step (such as reagent lots and step fields).
3. At the end of the run, NVCS sends one of the following run status requests:
   - RunCompletedSuccessfully
   - RunEndedByUSer
   - RunErroredOut

   The Sequencer API updates the step with any new information, which includes only the status and cycle/read information.
4. After primary analysis completes, NVCS sends a request containing the parsed run metrics. NVCS sends a request only when the run completed successfully, but the API does not make this assumption and accepts the request regardless of status. The Sequencer API then does the following actions:
   a. Records the metrics into the fields on file placeholder outputs in the LIMS.
   b. Completes the step in Clarity LIMS when the status is RunCompletedSuccessfully. For all other status options, the step remains in progress. This action is the default autocomplete step behavior. For details, refer to *NovaSeq 6000 Integration v3.5.1 Configuration and User Interaction* on page 369.

   > *NovaSeq 6000 Integration v3.5.1 Configuration and User Interaction* on page 369 provides detailed information on how the following components work with the Sequencer API integration:
   >
   > - Sample sheet generation and contents
   > - NovaSeq 6000 run
   > - User authentication and login
   > - Run recipe contents

Troubleshooting

If an automation trigger does not appear to run its corresponding scripts, refer to the following sections in the Clarity LIMS API documentation:

- *Troubleshooting Automated Informatics*
- *Troubleshooting Automation in the API*

If an error occurs that does not provide direction on how to proceed, complete the following steps:

1. Confirm the version of the installed Illumina NovaSeq 6000 Integration Package by running the following command on the Clarity LIMS server command line:

```
rpm -qa | grep -i novaseq
```

2. If the error is related to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7) step, review the log file information. For details, refer to *Logging* on page 448.

If the automated run step starts but does not complete, proceed as follows.

1. Use one of the following methods to open the in progress step in Clarity LIMS:
   - Log in to the default user account.
   - In Lab View, find the step in the Recent Activities pane.
   - Search for the step in Clarity LIMS, using either the Library Tube, or Flowcell barcode as the search term. The steps depend on whether the search is a NovaSeq Standard or NovaSeq Xp run.
2. On the Record Details screen, the Sequencing Log multiline text field contains logging information. If you are unable to reach the Record Details screen, or if the Sequencing Log field does not contain enough information to resolve the issue, review the `sequencer-api.log` file. For details, refer to *Logging* on page 448.
3. Contact Illumina Support. Provide the relevant information from the troubleshooting steps already performed.

Logging

In addition to updating the Sequencing Log multiline text field on the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.7) step, the Sequencer API writes a detailed log file to the following location:

`/opt/gls/clarity/tomcat/current/logs/sequencer-api.log`

Log messages include the Library Tube ID and Flowcell ID whenever the messages are related to a sequencing run-related request. In the LIMS workflows, these IDs are recorded as container names.

Some log messages are not directly related to a sequencing run request, such as downloading the sample sheet. In this case, the file LIMS ID and file name are included in log messages. The sample sheet downloaded by the sequencing system has a name that includes the sequencing container ID (Library Tube for Standard or Flowcell for Xp). The relevant container name search finds most the log

messages for this request. However, if all messages for downloading a file are found, the LIMS ID of the file must be known. The LIMS ID is found in the Clarity LIMS API and, for sample sheets used in a run, in the recipe response (also included in the log file).

Example lines from the log file:

```
2021-06-19 21:32:19.705 INFO --- [] SequencerAPIApplication : Started
SequencerAPIApplication in 7.026 seconds (JVM running for 15.964)
...
2021-06-19 21:34:44.564 INFO --- [Library Tube Id=NV1234567-LIB, Flow Cell
Id=RM123DSXX] LimsApiLookupUtil : Searching for containers with name 'NV1234567-
LIB' or 'RM123DSXX'
....
2021-06-19 21:35:27.158 INFO --- [] FileService : Successfully retrieved file
'NV1234567-LIB.csv' with LIMS ID 40-51 through the API.
```

NovaSeq 6000 Integration v3.5.1 Manual Upgrade

NovaSeq Integration Package v3.5.1 contains modifications to the existing automations from NovaSeq Integration Package v3.5.0.

If you do not want to upgrade the existing NovaSeq Sequencing workflow to the latest version through Illumina Preset Protocols (IPP), you can update the workflow configuration manually by completing the following steps.

Update Calculate Volumes Automation on Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.5.0) Step

Update the Calculate Volumes automation on the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.5.0) step as follows.

1. From Configuration, select the Automation tab.
2. Under the Step Automation tab, select the Calculate Volumes automation that is enabled on the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.5.0) master step.
3. Update the existing command line at the second -exp script as follows.
   Changes are highlighted in red.

```
-exp 'step.::PhiX Volume (ul):: = !step.hasValue(::% PhiX (2.5nM) Spike-In::) ?
0 : step.::% PhiX (2.5nM) Spike-In::; if (input.::Flowcell Type:: == ::SP::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 100;
step.::PhiX Volume (ul):: *= 0.6 }; if (input.::Flowcell Type:: == ::S1::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 100;
step.::PhiX Volume (ul):: *= 0.6 }; if (input.::Flowcell Type:: == ::S2::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 150;
step.::PhiX Volume (ul):: *= 0.9 }; if (input.::Flowcell Type:: == ::S4::) {
```


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```
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 310;
step.::PhiX Volume (ul):: *= 1.9 }; if (step.::PhiX Volume (ul):: == 0) {
step.::PhiX Volume (ul):: = :::: }; input.::Per Sample Volume (ul):: =
(((input.::Final Loading Concentration (pM):: * 5 / 1000) / input.::Normalized
Molarity (nM)::) * step.::Bulk Pool Volume (ul)::) / step.::Number of Samples in
Pool::' \
```

4. Update the existing command line at the fourth -exp script as follows.
   Changes are highlighted in red.

```
-exp 'if (step.::Total Sample Volume (ul):: >= step.::Bulk Pool Volume (ul)::)
{output.::RSB Volume (ul):: = 0} else {output.::RSB Volume (ul):: = step.::Bulk
Pool Volume (ul):: - step.::Total Sample Volume (ul)::} ; output.::Flowcell
Type:: = input.::Flowcell Type:: ; output.::Loading Workflow Type:: =
input.::Loading Workflow Type:: ; if ( input.::Flowcell Type:: == ::SP:: ) {
output.::Volume of Pool to Denature (ul):: = 100 ; output.::NaOH Volume (ul):: =
25 ; output.::Tris-HCl Volume (ul):: = 25 } ; if ( input.::Flowcell Type:: ==
::S1:: ) { output.::Volume of Pool to Denature (ul):: = 100 ; output.::NaOH
Volume (ul):: = 25 ; output.::Tris-HCl Volume (ul):: = 25 } ; if (
input.::Flowcell Type:: == ::S2:: ) {output.::Volume of Pool to Denature (ul)::
= 150 ; output.::NaOH Volume (ul):: = 37 ; output.::Tris-HCl Volume (ul):: = 38
} ; ; if ( input.::Flowcell Type:: == ::S4:: ) { output.::Volume of Pool to
Denature (ul):: = 310 ; output.::NaOH Volume (ul):: = 77 ; output.::Tris-HCl
Volume (ul):: = 78 }' \
```

5. Select Save.

Update Calculate Volumes Automation on Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.5.0) Step

Update the Calculate Volumes automation on the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.5.0) step as follows.

1. From Configuration, select the Automation tab.
2. Under the Step Automation tab, select the Calculate Volumes automation enabled on the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.5.0) step.
3. Update the existing command line at the second -exp script as follows.
   Changes to the existing command line are in red.

```
-exp 'step.::PhiX Volume (ul):: = !step.hasValue(::% PhiX (0.25nM) Spike-In::) ?
0 : step.::% PhiX (0.25nM) Spike-In::; if (input.::Flowcell Type:: == ::SP::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Lanes to Sequence:: * 18;
step.::PhiX Volume (ul):: *= 0.7 }; if (input.::Flowcell Type:: == ::S1::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Lanes to Sequence:: * 18;
step.::PhiX Volume (ul):: *= 0.7 }; if (input.::Flowcell Type:: == ::S2::) {
```


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```
step.::Bulk Pool Volume (ul):: = step.::Number of Lanes to Sequence:: * 22;
step.::PhiX Volume (ul):: *= 0.8 }; if (input.::Flowcell Type:: == ::S4::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Lanes to Sequence:: * 30;
step.::PhiX Volume (ul):: *= 1.1 }; if (step.::PhiX Volume (ul):: == 0) {
step.::PhiX Volume (ul):: = :::: }; input.::Per Sample Volume (ul):: =
(((input.::Final Loading Concentration (pM):: * 5 / 1000) / input.::Normalized
Molarity (nM)::) * step.::Bulk Pool Volume (ul)::) / step.::Number of Samples in
Pool::' \
```

4. Select Save.

Update Calculate Volumes Automation on Dilute, Denature & ExAmp (NovaSeq 6000 v3.5.0) Step

Update the Calculate Volumes automation on the Dilute, Denature & ExAmp (NovaSeq 6000 v3.5.0) step as follows.

1. From Configuration, select the Automation tab.
2. Under the Step Automation tab, select the Calculate Volumes automation enabled on the Dilute, Denature & ExAmp (NovaSeq 6000 v3.5.0) step.
3. Update the existing command line at the first -exp script as follows.
   Changes to the existing command line are in red.

```
-exp 'if ( input.::Flowcell Type:: == ::SP:: ) { output.::BP Aliquot Volume
(ul):: = 18 ; output.::NaOH Volume (ul):: = 4 ; output.::Tris-HCl Volume (ul)::
= 5 ; step.::DPX1 Volume (ul):: = 126 ; step.::DPX2 Volume (ul):: = 18 ;
step.::DPX3 Volume (ul):: = 66 ; output.::Mastermix per Lane (ul):: = 63} ; if (
input.::Flowcell Type:: == ::S1:: ) { output.::BP Aliquot Volume (ul):: = 18 ;
output.::NaOH Volume (ul):: = 4 ; output.::Tris-HCl Volume (ul):: = 5 ;
step.::DPX1 Volume (ul):: = 126 ; step.::DPX2 Volume (ul):: = 18 ; step.::DPX3
Volume (ul):: = 66 ; output.::Mastermix per Lane (ul):: = 63} ; if (
input.::Flowcell Type:: == ::S2:: ) { output.::BP Aliquot Volume (ul):: = 22 ;
output.::NaOH Volume (ul):: = 5 ; output.::Tris-HCl Volume (ul):: = 6 ;
step.::DPX1 Volume (ul):: = 126 ; step.::DPX2 Volume (ul):: = 18 ; step.::DPX3
Volume (ul):: = 66 ; output.::Mastermix per Lane (ul):: = 77} ; if (
input.::Flowcell Type:: == ::S4:: ) { output.::BP Aliquot Volume (ul):: = 30 ;
output.::NaOH Volume (ul):: = 7 ; output.::Tris-HCl Volume (ul):: = 8 ;
step.::DPX1 Volume (ul):: = 315 ; step.::DPX2 Volume (ul):: = 45 ; step.::DPX3
Volume (ul):: = 165 ; output.::Mastermix per Lane (ul):: = 105} ;
output.::Flowcell Type:: = input.::Flowcell Type:: ; output.::Loading Workflow
Type:: = input.::Loading Workflow Type::' \
```

4. Select Save.

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Update Validate Flowcell Barcode Automation on Load to Flowcell (NovaSeq 6000 v3.5.0) Step

Update the Validate Flowcell Barcode automation on the Load to Flowcell (NovaSeq 6000 v3.5.0) step as follows.

1. From Configuration, select the Automation tab.
2. Under the Step Automation tab, select the Validate Flowcell Barcode automation enabled on the Load to Flowcell (NovaSeq 6000 v3.5.0) step.
3. Update the existing command line as follows.
   Changes to the existing command line are in red.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-exp 'if (input.::Flowcell Type:: == ::SP:: && !output.container.name.matches( ::[A-Z0-9]{5}DR[A-Z0-9]{2}:: ) ) {fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: ) } ; if (input.::Flowcell Type:: == ::S1:: && !output.container.name.matches( ::[A-Z0-9]{5}DR[A-Z0-9]{2}:: ) ) {fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: ) } ; if (input.::Flowcell Type:: == ::S2:: && !output.container.name.matches( ::[A-Z0-9]{5}DM[A-Z0-9]{2}:: ) ) {fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: ) } ; if (input.::Flowcell Type:: == ::S4:: && !output.container.name.matches( ::[A-Z0-9]{5}DS[A-Z0-9]{2}:: ) ) {fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: ) } ; output.::Flowcell Type:: = input.::Flowcell Type:: ; output.::Loading Workflow Type:: = input.::Loading Workflow Type::' \
-log {compoundOutputFileLuid1}"
```

4. Select Save.

Update Validate Library Tube Barcode Automation on Dilute and Denature (NovaSeq 6000 v3.5.0) Step

Update the Validate Library Tube Barcode automation on the Dilute and Denature (NovaSeq 6000 v3.5.0) step as follows.

1. From Configuration, select the Automation tab.
2. Under the Step Automation tab, select the Validate Library Tube Barcode automation enabled on the Dilute and Denature (NovaSeq 6000 v3.5.0) step.
3. Update the existing command line as follows.
   Changes to the existing command line are in red.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
script:evaluateDynamicExpression -exp 'if (!output.container.name.matches( ::[A-
Z]{2}[0-9]{7}-[A-Z]{3}:: ) ) {fail ( ::Invalid Library Tube Barcode. Please
verify and try again.:: ) } ; output.::Flowcell Type:: = input.::Flowcell Type::
; output.::Loading Workflow Type:: = input.::Loading Workflow Type::' -log
{compoundOutputFileLuid1}"
```

4. Select Save.

NovaSeq 6000 Integration v3.5.1 Release Notes

These release notes describe the key changes to software components for the Clarity LIMS NovaSeq 6000 Integration Package.

Compatibility

This version of the integration package is compatible with the following software:

- Clarity LIMS v5.1 and later
- NGS Extensions Package v5.22 and later
- Illumina Preset Protocols (IPP) v2.5.0 and later
- NovaSeq Control Software (NVCS) v1.8.0
- Illumina bcl2fastq v2.20

New Features

Features provided with this release of the integration package are as follows.

- Updates to the Validate Flowcell Barcode automation (in the Load to Flowcell step of the NovaSeq Xp protocol) to support future flow cell barcode formats.
- Vulnerability fixes for Denial of Service (CVE-2022-22970) and Integer Overflow (CVE-2022-22976).

Defects Fixed

Fixed the following Calculate Volumes automation script values to sync with the *NovaSeq 6000 System Denature and Dilute Libraries Guide (document # 1000000106351)*:

- PhiX
- NaOH
- Tris-HCl
- Bulk pool volume

Known Issues

None

For Research Use Only. Not for use in diagnostic procedures.

NovaSeq 6000 Integration v3.5.0 Configuration and User Interaction

The Illumina NovaSeq 6000 Integration Package v3.5.0 supports the integration of Clarity LIMS to Illumina NovaSeq 6000 instruments. This integration supports Clarity v5.1 and later.

For instructions on validating and troubleshooting the Illumina NovaSeq 6000 Integration, refer to *NovaSeq 6000 Integration v3.5.0 Validation and Troubleshooting* on page 514.

The configuration provided in this integration has been established to support NovaSeq 6000 lab processes. Any configuration changes to protocols or workflows - including renaming protocols, steps, and fields - could break process.

Overview

The integration includes the following features:

- Preconfigured NovaSeq 6000 v3.5 workflow that maps to lab protocols and instrument runs.
- The following preconfigured protocols:
  - Run Format (NovaSeq 6000 v3.5)
  - NovaSeq Standard (NovaSeq 6000 v3.5) (supports the loading of pooled libraries into a library tube)
  - NovaSeq Xp (NovaSeq 6000 v3.5) (supports individual lane loading on the NovaSeq)
  - AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5)
- Automated generation of v1 sample sheet for use with bcl2fastq2 v2.20 analysis software or v2 sample sheet. This file is automatically uploaded to the instrument via the API.
- Automatic validation of run setup information. This information is used to create the run recipe and initiate the run.
- Automated tracking of the NovaSeq sequencing run and parsing of run statistics into Clarity LIMS, including the following features:
  - Progress and metrics of sequencing run
  - Per-instrument sequencing runs (tracked as part of the Run ID field value)
  - Sequencing run parameters
  - Sequencing run data directory location
  - Real-Time Analysis v3 (RTA3) version and other run-specific information
  - [Optional] Preconfigured Library Prep Validation v2.2.0 workflow used for validation purposes only. The workflow contains a single-step protocol that models the library prep required to

produce normalized libraries that are ready for the NovaSeq 6000 v3.5 workflow. For more information, refer to *NovaSeq 6000 Integration v3.5.0 Validation and Troubleshooting* on page 514. The Library Prep Validation v2.2.0 workflow is available in IPP v2.4 Package.

Prerequisites and Assumptions

It is assumed that samples enter the NovaSeq 6000 v3.5 workflow as normalized libraries. It is assumed that the following steps have completed before samples are assigned to the workflow:

- Samples have been accessioned into the Clarity LIMS.
- Samples have been run through QC and library prep.
- Samples have been normalized, and the value is captured in a field called Normalized Molarity (nM).

For more information on sample accessioning, refer to *Sample Accessioning* and *Upload and Modify Samples* in the Getting Started section of the Clarity LIMS Core Functionality documentation.

You can assign samples to workflows automatically, using a routing script, or manually—from the Projects & Samples dashboard. Refer to *Assign and Process Samples* in the Clarity LIMS Core Functionality documentation.

Workflows, Protocols, and Steps

The Illumina NovaSeq 6000 Integration Package v3.5.0 includes the following workflows:

- NovaSeq 6000 v3.5
- Library Prep Validation v2.2.0 (optional, but recommended for validation purposes)

The following table describes the protocols and steps included in these workflows.

Table 138 NovaSeq 6000 Integration Package v3.5.0 Protocols and Steps

Protocol Name	Protocol Purpose	Steps Included in Protocol
Library Prep Validation v2.2.0 Workflow		
Library Prep Validation v2.2.0	Included for validation purposes only, this protocol models the library prep steps required to advance samples to the Run Format (NovaSeq 6000 v3.5) protocol. The protocol contains a single step - Library Prep Validation v2.2.0. After this step, a routing script sends the samples to the first step of the NovaSeq 6000 v3.5 workflow - Define Run Format (NovaSeq 6000 v3.5).	1. Library Prep Validation v2.2.0
NovaSeq 6000 v3.5 Workflow		

Protocol Name	Protocol Purpose	Steps Included in Protocol
Run Format (NovaSeq 6000 v3.5)	Allows for the assignment of per sample values for: • Loading Workflow Type: Select either NovaSeq Standard or NovaSeq Xp. • Normalized Molarity: Enter a value for each sample. • Flowcell Type: Select from options SP, S1, S2, or S4. • Final Loading Concentration (pM): Select from options 225 (PCR-free workflows) or 400 (Nano workflows), or enter a different value. Compares the Normalized Molarity value of each sample with the Minimum Molarity value. Routing script sends samples to the NovaSeq Standard or NovaSeq Xp protocol, according to the selected Loading Workflow Type. Samples with Normalized Molarity less than Minimum Molarity are removed from the workflow.	1. Define Run Format (NovaSeq 6000 v3.5)
NovaSeq Standard (NovaSeq 6000 v3.5)	Samples are pooled and added to the library tube in preparation for the NovaSeq run. The run setup information is validated and a sample sheet is generated. Routing script sends the library tube to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5) protocol.	1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.5) 2. Dilute and Denature (NovaSeq 6000 v3.5)
NovaSeq Xp (NovaSeq 6000 v3.5)	Samples are pooled and added to lanes on the NovaSeq flow cell. The flow cell type is determined by the option selected in the Define Run Format (NovaSeq 6000 v3.5) step. The run setup information is validated and a sample sheet is generated. Routing script sends flow cell to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5) protocol.	1. Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.5) 2. Dilute, Denature, and ExAmp (NovaSeq 6000 v3.5) 3. Load to Flowcell (NovaSeq 6000 v3.5)

Protocol Name	Protocol Purpose	Steps Included in Protocol
AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5)	All samples complete the workflow by going through this protocol. This protocol contains one fully automated step. Do not add samples to the Ice Bucket or start the step. The integration starts the step automatically.	1. AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5)

Validation Workflow

The Library Prep Validation v2.2.0 workflow allows for validation of the system after installation is complete. For details, refer to *NovaSeq 6000 Integration v3.5.0 Validation and Troubleshooting* on page 514.

Step Automations

The following table lists the step automations included in the NovaSeq 6000 v3.5 workflow. Automation details are provided in the documentation for each step.

For details on the validation workflow, refer to *NovaSeq 6000 Integration v3.5.0 Validation and Troubleshooting* on page 514.

Table 139 NovaSeq 6000 v3.5 Workflow Step Automations

Protocol	Step	Automation
1. Run Format (NovaSeq 6000 v3.5)	1. Define Run Format (NovaSeq 6000 v3.5)	Routing Script Set Next Steps

Protocol	Step	Automation
2. NovaSeq Standard (NovaSeq 6000 v3.5)	1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.5)	Calculate Volumes Set Next Step Validate Inputs Flowcell Type and Single Pool Validate Unique Indexes (Not used. This functionality is handled by the core Clarity LIMS configuration for pooling.)
	2. Dilute and Denature (NovaSeq 6000 v3.5)	Routing Script Check Library Tube Barcode Validate Run Setup and Generate Sample Sheet Validate Single Input
3. NovaSeq Xp (NovaSeq 6000 v3.5)	1. Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.5)	Calculate Volumes Set Next Step Validate Inputs Flowcell Type and Single Pool Validate Unique Indexes (Not used. This functionality is handled by the core Clarity LIMS configuration for pooling.)
	2. Dilute, Denature, and ExAmp (NovaSeq 6000 v3.5)	Calculate Volumes Set Next Step Validate Inputs Flowcell Type
	3. Load to Flowcell (NovaSeq 6000 v3.5)	Validate Flowcell Barcode Validate Inputs and Selected Container Validate Run Setup and Generate Sample Sheet
4. AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5)	1. AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5)	Update Lane Number Set Next Steps (Not used. By default, this functionality is determined by the Sequencer API. If necessary, enable the automation and use it to override the next step behavior used by the Sequencer API.)

Terminology

This integration package supports BaseSpace Clarity LIMS v5.1 and later.

As of Clarity LIMS v5, the term user-defined field (UDF) has been replaced with custom field in the user interface. However, the API resource is still called UDF.

There are two types of custom fields:

- Master step fields—Configured on master steps. Master step fields only apply to the following:
  - The master step on which the fields are configured.
  - The steps derived from those master steps.
- Global fields—Configured on entities (eg, submitted sample, derived sample, measurement, etc.). Global fields apply to the entire Clarity LIMS system.

Protocol 1: Run Format (NovaSeq 6000 v3.5)

This protocol sets the Loading Workflow Type and allows the choice of the appropriate Flowcell Type and Final Loading Concentration (pM). After the protocol, a routing script sends the normalized libraries to either the NovaSeq Standard (NovaSeq 6000 v3.5) or the NovaSeq Xp (NovaSeq 6000 v3.5) protocol.

This protocol contains one step: Define Run Format (NovaSeq 6000 v3.5).

Step 1. Define Run Format (NovaSeq 6000 v3.5)

Step input: NTP (normalized libraries)

Step output: None

Set Next Steps Automation

Automatically triggered on exit of the Record Details screen, this automation completes the following actions:

- Sets the next step for samples to REMOVE:

```
nextStep = ::REMOVE::
```

- Calculates the Minimum Molarity using the following formula:

```
input.::Minimum Molarity (nM):: = (5 * input.::Final Loading Concentration
(pM)::)/1000
```

- Checks Normalized Molarity value. For samples with no Normalized Molarity value (ie, empty value, not including 0), it generates an error message informing that the field cannot be empty:

```
if (!input.hasValue(::Normalized Molarity (nM)::)) { fail(::The Normalized
Molarity cannot be empty.::) ; }
```

- Compares each sample Normalized Molarity value with the Minimum Molarity value. If Normalized Molarity value is lower than the Minimum Molarity value, it sets the samples Loading Workflow Type to [Remove from workflow] and records a message in the Warning field for the sample:

```
else if (input.::Normalized Molarity (nM):: < input.::Minimum Molarity (nM)::) {
input.::Warning:: = ::The Normalized Molarity is too low.:: ;
input.::Loading Workflow Type:: = ::[Remove from workflow]:: ; }
else { input.::Warning:: = ::n/a:: }
```

At this point there are two options:

- Correct the Normalized Molarity value on the Record Details screen. Edit the Loading Workflow Type field and set it to NovaSeq Standard or NovaSeq Xp, as applicable.
- Complete the protocol without correcting the Normalized Molarity value. In this case, those samples are removed from the workflow.

Routing Script Automation

Automatically triggered on exit of the step, this automation invokes the changeWorkflow script, which routes step inputs appropriately.

- Samples with Loading Workflow Type field value = NovaSeq Standard are routed to the NovaSeq 6000 v3.5 workflow and queued for the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.5) step.
- Samples with Loading Workflow Type field value = NovaSeq Xp are routed to the NovaSeq 6000 v3.5 workflow and queued for the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.5) step.

Default automation command line is as follows:

```
bash -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar
-u {username} -p {password} -i {stepURI:v2} -l {compoundOutputFileLuid0}
script:changeWorkflow \
\
--FIELD_NAME 'Loading Workflow Type' \
--FIELD_VALUE 'NovaSeq Standard' \
--WORKFLOW 'NovaSeq 6000 v3.5' \
--STEP 'Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.5)' \
--INPUTS_OR_OUTPUTS 'INPUTS' \
\
--FIELD_NAME 'Loading Workflow Type' \
--FIELD_VALUE 'NovaSeq Xp' \--WORKFLOW 'NovaSeq 6000 v3.5' \
--STEP 'Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.5)' \
--INPUTS_OR_OUTPUTS 'INPUTS'"
```


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Master Step Fields

The following table lists field configuration details defined on the Define Run Format (NovaSeq 6000 v3.5) master step.

Table 140 Define Run Format (NovaSeq 6000 v3.5) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Comment	Multiline Text		None
Flowcell Type	Text Dropdown	Required Field Custom Entries	Presets • SP • S1 • S2 • S4
Instruction	Text	Read Only	Default • Add Flowcell Type and Loading Workflow Type below
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp

Global Fields

The following table lists the global custom fields that are configured to display on the Define Run Format (NovaSeq 6000 v3.5) step.

Table 141 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
Adjusted Per Sample Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Final Loading Concentration (pM)	Numeric Dropdown	Required Field Custom Entries	Decimal places displayed = 0 Presets • 225 • 400

Field Name	Field Type	Options	Additional Options and Dropdown Items
Flowcell Type	Text Dropdown	Required Field	Presets • SP • S1 • S2 • S4
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
Minimum Molarity (nM)	Numeric		Decimal places displayed = 2
Normalized Molarity (nM)	Numeric		Decimal places displayed = 2
Per Sample Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Warning	Text Dropdown	Read Only Custom Entries	The Normalized Molarity (nM) is too low. Not applicable

User Interaction

Refer to Run Define Run Format (NovaSeq 6000 v3.5) step section in *NovaSeq 6000 Integration v3.5.0 Validation and Troubleshooting* on page 514.

Protocol 2: NovaSeq Standard (NovaSeq 6000 v3.5)

Samples are routed to this protocol if their Loading Workflow Type value is set to NovaSeq Standard. Samples are pooled and added to a library tube in preparation for the NovaSeq run.

At the end of this protocol, a routing script sends the library tube to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5) protocol.

This protocol contains two steps:

- Step 1: Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.5)
- Step 2: Dilute and Denature (NovaSeq 6000 v3.5)

Step 1: Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.5)

In this step, libraries are placed manually into a single pool. Resuspension buffer and reagents are added.

Create only one pool per step.

Step input: NTP (normalized libraries)

Step output: Bulk pool

Validate Unique Indexes automation is not used. This functionality is handled by the core Clarity LIMS configuration for pooling.

Validate Inputs Flowcell Type and Single Pool Automation

Automatically triggered on exit of the Pooling screen, this automation completes the following actions:

- Checks that all samples in the pool have the same Flowcell Type assigned to them.
- Checks that only one pool has been created.
- Changes the value of error and logging messages to reference the type of pool passed as bulk pool.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar \
script:validate_flowcell_for_input_pools \
  -i {stepURI:v2} \
  -u {username} \
  -p {password} \
  -l {compoundOutputFileLuid1} \
  -validateSingleOutput true \
  -poolType bulk"
```

Calculate Volumes Automation

Automatically triggered when selecting the Calculate Volumes button on the Record Details screen, this automation completes the following actions:

- Calculates the number of samples in the pool.

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: + 1
```

- Sets the value of the Bulk Pool Volume (µl), and PhiX Volume (µl) field based on the selected Flowcell Type.

```
step.::PhiX Volume (ul):: = !step.hasValue(::% PhiX (2.5nM) Spike-In::) ? 0 :
step.::% PhiX (2.5nM) Spike-In::;
if (input.::Flowcell Type:: == ::SP::) {
```


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```
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 100
+ 30;
step.::PhiX Volume (ul):: *= 0.8
};
if (input.::Flowcell Type:: == ::S1::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 100
+ 30;
step.::PhiX Volume (ul):: *= 0.8
};
if (input.::Flowcell Type:: == ::S2::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 150
+ 30;
step.::PhiX Volume (ul):: *= 1.1
};
if (input.::Flowcell Type:: == ::S4::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 310
+ 30;
step.::PhiX Volume (ul):: *= 2.1
};
if (step.::PhiX Volume (ul):: == 0) {
step.::PhiX Volume (ul):: = ::::
};
```

- Calculates the Per Sample Volume (µl) to be added to the pool.

```
input.::Per Sample Volume (ul):: = ( ( (input.::Final Loading Concentration
(pM):: * 5 / 1000) / input.::Normalized Molarity (nM):: ) * step.::Bulk Pool
Volume (ul):: ) / step.::Number of Samples in Pool::
```

> To ensure accurate pipetting of each sample in a pool for sequencing, the Per Sample Volume (µl) value must be equal to or higher than the Minimum Per Sample Volume (µl) (default value, set at 5, may be edited).
>
> Assuming the default Minimum Per Sample Volume (µl) value of 5, completes the following steps for a given batch:
>
> 1. If the smallest Per Sample Volume (µl) value is less than 5, automatically assigns a value of 5 to the sample Adjusted Per Sample Volume (µl) field.
> 2. Adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

- Calculates the Total Sample Volume (µl) field value.

```
step.::Total Sample Volume (µl):: = step.::Total Sample Volume (µl):: +
input.::Adjusted Per Sample Volume (µl)::
```


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- If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) field value.

```
if (step.::Total Sample Volume (ul):: >= step.::Bulk Pool Volume (ul)::)
{output.::RSB Volume (ul):: = 0}
else {output.::RSB Volume (ul):: = step.::Bulk Pool Volume (ul):: - step.::Total
Sample Volume (ul)::} ;
```

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs.

```
output.::Flowcell Type:: = input.::Flowcell Type:: ;
output.::Loading Workflow Type:: = input.::Loading Workflow Type:: ;
```

- Sets the Volume of Pool to Denature (µl) value and calculates the NaOH Volume (µl) and Tris-HCl Volume (µl) values based on the Flowcell Type:

```
if ( input.::Flowcell Type:: == ::SP:: ) { output.::Volume of Pool to Denature
(ul):: = 100 ; output.::NaOH Volume (ul):: = 25 ; output.::Tris-HCl Volume
(ul):: = 25 } ;
if ( input.::Flowcell Type:: == ::S1:: ) { output.::Volume of Pool to Denature
(ul):: = 100 ; output.::NaOH Volume (ul):: = 25 ; output.::Tris-HCl Volume
(ul):: = 25 } ;
if ( input.::Flowcell Type:: == ::S2:: ) { output.::Volume of Pool to Denature
(ul):: = 150 ; output.::NaOH Volume (ul):: = 37.50 ; output.::Tris-HCl Volume
(ul):: = 37.50 } ;
if ( input.::Flowcell Type:: == ::S4:: ) { output.::Volume of Pool to Denature
(ul):: = 310 ; output.::NaOH Volume (ul):: = 77.50 ; output.::Tris-HCl Volume
(ul):: = 77.50 }
```

- Uses the `NovaSeq_Standard_Bulk_Pool1.csv`, `NovaSeq_Standard_Bulk_Pool2.csv`, and `NovaSeq_Standard_Bulk_Pool3.csv` template files to generate a single CSV file containing information about the pool and the samples it contains. The generated file is available for download on the Step Setup screen of Dilute and Denature (NovaSeq 6000 v3.5).

```
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Standard_Bulk_
Pool1.csv \
-o 1.csv \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Standard_Bulk_
Pool2.csv \
-o 2.csv \
```

```
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Standard_Bulk_
Pool3.csv \
-o 3.csv \
&& cat 1.csv 2.csv 3.csv > {compoundOutputFileLuid0}.csv
```

- Resets the Total Sample Volume (µl) and Number of Samples in Pool field values so that the automation is idempotent.

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: - 1;
step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: -
input.::Adjusted Per Sample Volume (ul)::
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, the following automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol, Dilute, and Denature (NovaSeq 6000 v3.5):

```
nextStep = ::ADVANCE::
```

Master Step Fields

The following table lists configuration details defined on the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.5) step.

Table 142 Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.5) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
% PhiX (2.5 nM) Spike-In	Numeric		Range = 0–100 Decimal places displayed = 0
Bulk Pool Volume (µl) (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 2
Number of Flowcells to Sequence	Numeric	Required Field	Range = 1–10 Decimal places displayed = 0

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Field Name	Field Type	Options	Additional Options and Dropdown Items
Minimum Per Sample Volume (µl)	Numeric	Required Field	Decimal places displayed = 2 Default • 5
Number of Samples in Pool (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 0 Default • 0
PhiX Volume (µl) (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 2
Total Sample Volume (µl) (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 2 Default • 0

Global Fields

The following table lists the global custom fields that are configured to display on the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.5) step.

Table 143 Global Custom Fields Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
Flowcell Type	Text Dropdown	Required Field	Presets • SP • S1 • S2 • S4
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

Field Name	Field Type	Options	Additional Options and Dropdown Items
NaOH Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
RSB Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Tris-HCl Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Volume of Pool to Denature (µl) Used in Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.5) step only. Displays on Record Details screen and in the generated CSV file.	Numeric	Read Only	Decimal places displayed = 0

User Interaction

Refer to Run Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.5) step section in *NovaSeq 6000 Integration v3.5.0 Validation and Troubleshooting* on page 514.

Step 2: Dilute and Denature (NovaSeq 6000 v3.5)

In this step, pooled samples are denatured and diluted by the addition of NaOH, Tris-HCl, and Resuspension Buffer (RSB). Manually place the pooled samples into the library tube for the NovaSeq run.

In addition, this step validates the run setup information and generates the sample sheet file.

Step input: Bulk pool

Step output: Library tube

Validate Single Input Automation

Automatically triggered at the beginning of the step, this automation checks that there is only one container input to the step.

```
script:validateSampleCount -min 1 -max 1
```

Validate Library Tube Barcode Automation

Automatically triggered on exit of the Placement screen, this automation completes the following actions:

- Validates the library tube barcode to ensure it conforms to the barcode mask [A-Z]{2}[0-9]{7}-[A-Z]{3}:

```
if (!output.container.name.matches(::[A-Z]{2}[0-9]{7}-[A-Z]{3}::)) {
fail(::Invalid Library Tube Barcode. Verify and try again.::)
}
```

- Copies Flowcell Type and Loading Workflow Type field values from step inputs to outputs.

```
output.::Flowcell Type:: = input.::Flowcell Type:: ;
output.::Loading Workflow Type:: = input.::Loading Workflow Type::
```

Validate Run Setup and Generate Sample Sheet Automation

The script definition portion of Validate Run Setup has been moved into the Validation Script custom field due to the 4000 character limit.

- If modification is required on Validate Run Setup script, perform the changes directly on the Validation Script custom field default value.
- The script definition portion of the Generate Sample Sheet remains untouched.

Triggered by a button on the Record Details screen, this automation completes the following actions:

- Copies the Flowcell Type from the step input to the Run Mode field (hidden):

```
step.::Run Mode:: = input.::Flowcell Type::
```

- Validates the parameters entered on the Record Details screen, which are used to set up the run and generate the sample sheet file. For more information, refer to the *Respond to Validation / Recipe Request Call from Instrument* section of *NovaSeq 6000 Run* on page 501.
  - Experiment Name may only contain alphanumeric, dash, or underscore characters. Spaces are not permitted.
  - When Workflow Type = No Index, both Index Read 1 and Index Read 2 must be zero.
  - When Workflow Type = Single Index, Index Read 1 must be greater than zero, and Index Read 2 must be zero.
  - When Workflow Type = Dual Index, both Index Read 1 and Index Read 2 must be greater than zero.

```
if(!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) {
fail(::Experiment Name contains prohibited characters. Allowed characters are:
a-z, A-Z, 0-9, -, and _::)
};
if (step.::Workflow Type:: == ::No Index::) {
```

```
if (step.::Index Read 1:: != 0 || step.::Index Read 2:: != 0) {
fail(::Index Read 1 and Index Read 2 must be 0 if No Index is selected.::)
}
} else if (step.::Workflow Type:: == ::Single Index::) {
if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) {
fail(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if Single
Index is selected.::)
}
} else {
if (step.::Index Read 1:: == 0 || step.::Index Read 2:: == 0) {
fail(::Index Read 1 and Index Read 2 must be greater than 0 if Dual Index is
selected.::)
}
};
```

- Checks the Paired End and Read 2 Cycles field values.
  - If Paired End = True and Read 2 Cycles value is zero, generates an error.
  - If Paired End = False, Read 2 Cycles must be zero and UMI - Read 2 Length and UMI - Read 2 Start From Cycle must be empty.

```
if (step.::Paired End::.toBoolean()) {
if (step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) {
fail(::Read 1 Cycles and Read 2 Cycles must be greater than 0 if Paired End is
True.::)
}
} else {
if (step.::Read 2 Cycles:: != 0) {
fail(::Read 2 Cycles must be 0 if Paired End is False.::)
};
if (step.hasValue(::UMI - Read 2 Length::) || step.hasValue(::UMI - Read 2 Start
From Cycle::)) {
fail(::UMI - Read 2 Length and UMI - Read 2 Start From Cycle cannot be defined
if Paired End is False.::)
}
};
```

- Checks the Flowcell field value.
  - If Flowcell Type value is not SP, checks that the values of Read 1 Cycles and Read 2 Cycles are each 151 or less. If the value is greater than 151, an error message is generated.

```
if (input.::Flowc ell Type::!=::SP:: && step.::Read 1 Cycles:: > 151) {
fail(::Read 1 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
};
```

```
if (input.::Flowcell Type::!=::SP:: && step.::Read 2 Cycles:: > 151) {
fail(::Read 2 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
};
```

- Validates UMI Settings.
  - If UMI - Read 1 Length has value, then UMI - Read 1 Start From Cycle must have value.
  - If UMI - Read 1 Length has no value, then UMI - Read 1 Start From Cycle must not have value.
  - If UMI - Read 2 Length has value, then UMI - Read 2 Start From Cycle must have value.
  - If UMI - Read 2 Length has no value, then UMI - Read 2 Start From Cycle must not have value.
  - If UMI - Read 1 Length has no value, then UMI - Read 2 Length must not have value.
  - If UMI - Read 1 Start From Cycle has no value, then UMI - Read 2 Start From Cycle must not have value.

```
if (step.hasValue(::UMI - Read 1 Length::) && !step.hasValue(::UMI - Read 1
Start From Cycle::)) {
fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 1
Length is greater than 0.::)
};
if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 1
Start From Cycle::)) {
fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 1 Start From
Cycle is greater than 0.::)
};
if (step.hasValue(::UMI - Read 2 Length::) && !step.hasValue(::UMI - Read 2
Start From Cycle::)) {
fail(::UMI - Read 2 Start From Cycle must be greater than 0 if UMI - Read 2
Length is greater than 0.::)
};
if (!step.hasValue(::UMI - Read 2 Length::) && step.hasValue(::UMI - Read 2
Start From Cycle::)) {
fail(::UMI - Read 2 Length must be greater than 0 if UMI - Read 2 Start From
Cycle is greater than 0.::)
};
if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 2
Length::)) {
fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 2 Length is
greater than 0.::)
};
if (!step.hasValue(::UMI - Read 1 Start From Cycle::) && step.hasValue(::UMI -
Read 2 Start From Cycle::)) {
```


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```
fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 2
Start From Cycle is greater than 0.::)
};
```

- Validate Analysis Software Version to ensure it is a valid version string.

```
if (step.hasValue(::Analysis Software Version::) && !step.::Analysis Software
Version::.matches(::^(\\d+)(\\.\\d+)*$::)) {
fail(::Analysis Software Version contains prohibited characters. Allowed
characters are 0-9 and period. It shall start and end with numbers and separated
by single period e.g. 3.8.4::)
};
```

- Validate Override Cycles value to only contain Y, N, I, U, 0-9, and semicolon characters.

```
if (step.hasValue(::Override Cycles::) && !step.::Override Cycles::.matches(::
[YNIU0-9;]+::)) {
fail(::Override Cycles contains prohibited characters. Allowed characters are:
Y, N, I, U, 0-9 and ;. Example: N1Y150;I8;I7N1;Y141U10.::)
};
```

- Outputs the Settings Header in sample sheet if UMI - Read 1 Length or UMI - Read 2 Length have value.

```
step.::Settings Header:: = step.hasValue(::UMI - Read 1 Length::) ||
step.hasValue(::UMI - Read 2 Length::) ? ::[Settings]:: : ::::;
```

- Sets the next step for samples to REMOVE.

```
nextStep = ::REMOVE::
```

- Generates the sample sheet and attaches it to the step. For more information, refer to *Sample Sheet Generation* on page 500. Sample sheet template used depends on the selected value of Samplesheet Format and/or Reverse Complement Workflow.

```
&& if [ '{udf:Samplesheet Format}' == 'V2' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Samplesheet_v2.csv'; \
elif [ '{udf:Reverse Complement Workflow}' == 'true' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Reverse_Complement_Samplesheet.csv'; \
elif [ '{udf:Reverse Complement Workflow}' == 'false' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Samplesheet.csv'; \
else echo 'UDF Reverse Complement Workflow is empty or undefined.'; exit -1; fi
\
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/$templateFilename \
-o {compoundOutputFileLuid0}.csv \
```

```
-q true \
-destLIMSID {compoundOutputFileLuid0} \
-l {compoundOutputFileLuid1}"
```

Routing Script Automation

Automatically triggered on exit of the step, this automation invokes the changeWorkflow script, which routes step outputs to the NovaSeq 6000 v3.5 workflow and queues them for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5) step.

Default automation command line is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar
-u {username} -p {password} -i {stepURI:v2} -l {compoundOutputFileLuid2}
script:changeWorkflow \
--FIELD_NAME 'N/A' \
--FIELD_VALUE 'N/A' \
--WORKFLOW 'NovaSeq 6000 v3.5' \
--STEP 'AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5)' \
--INPUTS_OR_OUTPUTS 'OUTPUTS'"
```

Master Step Fields

The following table lists field configuration details cdefined on the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.5) step. These fields are required for sample sheet and JSON file generation.

Table 144 Dilute and Denature (NovaSeq 6000 v3.5) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Analysis Software Version	Text		Only applicable for v2 sample sheet. Otherwise, leave this field blank.
BaseSpace Sequence Hub Configuration	Text Dropdown	Required Field	Presets • Not Used • Run Monitoring Only • Run Monitoring and Storage

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Field Name	Field Type	Options	Additional Options and Dropdown Items
Custom Recipe Path	Text		
Experiment Name	Text	Required Field	
Index Read 1	Numeric Dropdown	Required Field Custom Entries	Range = 0–20 Decimal places displayed = 0 Presets • 0 • 6 • 8
Index Read 2	Numeric Dropdown	Required Field Custom Entries	Range = 0–20 Decimal places displayed = 0 Presets • 0 • 6 • 8
Output Folder	Text	Required Field	
Override Cycles	Text		Only applicable for v2 sample sheet. Otherwise, leave this field blank.
Paired End	Text Dropdown	Required Field	Presets • True • False
Read 1 Cycle	Numeric Dropdown	Required Field Custom Entries	Range = 1–251 Decimal places displayed = 0 Presets • 251* • 151 • 101 • 51 *Value of 251 is only supported for SP flow cell type. For all other cell types, maximum value is 151.

For Research Use Only. Not for use in diagnostic procedures.

Field Name	Field Type	Options	Additional Options and Dropdown Items
Read 2 Cycle	Numeric Dropdown	Required Field Custom Entries	Range = 0–251 Decimal places displayed = 0 Presets • 251* • 151 • 101 • 51 *Value of 251 is only supported for SP flow cell type. For all other cell types, maximum value is 151.
Reverse Complement Workflow	Toggle Switch	Required Field	Default • Yes
Run Mode (Not displayed in user interface)	Text Dropdown	Read Only	Presets • SP • S1 • S2 • S4
Samplesheet Format	Text Dropdown	Required Field	Presets • V1 (default) • V2
Setting Header (not displayed in user interface)	Text	Read Only	
UMI - Read 1 Length	Numeric	Custom Entries	Range = 1
UMI - Read 1 Start From Cycle	Numeric	Custom Entries	Range = 1
UMI - Read 2 Length	Numeric	Custom Entries	Range = 1
UMI - Read 2 Start From Cycle	Numeric	Custom Entries	Range = 1
Use Custom Index Read 1 Primer	Toggle Switch		Default • None Set

For Research Use Only. Not for use in diagnostic procedures.

Field Name	Field Type	Options	Additional Options and Dropdown Items
Use Custom Read 1 Primer	Toggle Switch		Default • None Set
Use Custom Read 2 Primer	Toggle Switch		Default • None Set
Use Custom Recipe	Toggle Switch	Required Field	Default • None Set
Validation Script	Multiline Text	Required Field Read Only	Default value is provided in the drop-down section that follows the table. Do not remove this field as it is used by Validate Run Setup and Generate Sample Sheet automation script.
Workflow	Text	Read Only	Default • GenerateFASTQ
Workflow Type	Text Dropdown	Required Field	Presets • No Index • Single Index • Dual Index • Custom

Validation Script

```
step.::Run Mode:: = input.::Flowcell Type::; if (!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) { fail(::Experiment Name contains prohibited characters. Allowed characters are: a-z, A-Z, 0-9, -, and _::) }; if (step.::Workflow Type:: == ::No Index::) { if (step.::Index Read 1:: != 0 || step.::Index Read 2:: != 0) { fail(::Index Read 1 and Index Read 2 must be 0 if No Index is selected.::) } } else if (step.::Workflow Type:: == ::Single Index::) { if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) { fail(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if Single Index is selected.::) } } else { if (step.::Index Read 1:: == 0 || step.::Index
```

```
Read 2:: == 0) { fail(::Index Read 1 and Index Read 2 must be greater than 0 if
Dual Index is selected.::) } }; if (step.::Paired End::.toBoolean()) { if
(step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) { fail(::Read 1
Cycles and Read 2 Cycles must be greater than 0 if Paired End is True.::) } }
else { if (step.::Read 2 Cycles:: != 0) { fail(::Read 2 Cycles must be 0 if
Paired End is False.::) }; if (step.hasValue(::UMI - Read 2 Length::) ||
step.hasValue(::UMI - Read 2 Start From Cycle::)) { fail(::UMI - Read 2 Length
and UMI - Read 2 Start From Cycle cannot be defined if Paired End is False.::) }
}; if (input.::Flowcell Type:: != ::SP:: && step.::Read 1 Cycles:: > 151) { fail
(::Read 1 Cycles must not be larger than 151 if it is not SPrime Flowcell::) };
if (input.::Flowcell Type:: != ::SP:: && step.::Read 2 Cycles:: > 151) { fail
(::Read 2 Cycles must not be larger than 151 if it is not SPrime Flowcell::) };
if (step.hasValue(::UMI - Read 1 Length::) && !step.hasValue(::UMI - Read 1
Start From Cycle::)) { fail(::UMI - Read 1 Start From Cycle must be greater than
0 if UMI - Read 1 Length is greater than 0.::) }; if (!step.hasValue(::UMI -
Read 1 Length::) && step.hasValue(::UMI - Read 1 Start From Cycle::)) { fail
(::UMI - Read 1 Length must be greater than 0 if UMI - Read 1 Start From Cycle
is greater than 0.::) }; if (step.hasValue(::UMI - Read 2 Length::) &&
!step.hasValue(::UMI - Read 2 Start From Cycle::)) { fail(::UMI - Read 2 Start
From Cycle must be greater than 0 if UMI - Read 2 Length is greater than 0.::)
}; if (!step.hasValue(::UMI - Read 2 Length::) && step.hasValue(::UMI - Read 2
Start From Cycle::)) { fail(::UMI - Read 2 Length must be greater than 0 if UMI
- Read 2 Start From Cycle is greater than 0.::) }; if (!step.hasValue(::UMI -
Read 1 Length::) && step.hasValue(::UMI - Read 2 Length::)) { fail(::UMI - Read
1 Length must be greater than 0 if UMI - Read 2 Length is greater than 0.::) };
if (!step.hasValue(::UMI - Read 1 Start From Cycle::) && step.hasValue(::UMI -
Read 2 Start From Cycle::)) { fail(::UMI - Read 1 Start From Cycle must be
greater than 0 if UMI - Read 2 Start From Cycle is greater than 0.::) };
step.::Settings Header:: = step.hasValue(::UMI - Read 1 Length::) ||
step.hasValue(::UMI - Read 2 Length::) ? ::[Settings]:: : ::::; nextStep =
::REMOVE::
```

Global Fields

The following table lists the global custom fields that are configured to display on the Dilute and Denature (NovaSeq 6000 v3.5) step.

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Table 145 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
Flowcell Type	Text Dropdown	Required Field	Presets • SP • S1 • S2 • S4
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User Interaction

Refer to Run Dilute and Denature (NovaSeq 6000 v3.5) step section in *NovaSeq 6000 Integration v3.5.0 Validation and Troubleshooting* on page 514.

Protocol 3: NovaSeq Xp (NovaSeq 6000 v3.5)

Samples are routed to this protocol if their Loading Workflow Type value is set to NovaSeq Xp.

Samples are pooled and added to lanes on the NovaSeq flow cell type selected in the Define Run Format (NovaSeq 6000 v3.5) step. At the end of this protocol, the flow cell is sent to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5) protocol.

This protocol contains the following three steps:

- Step 1: Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.5)
- Step 2: Dilute, Denature, and ExAmp (NovaSeq 6000 v3.5)
- Step 3: Load to Flowcell (NovaSeq 6000 v3.5)

Step 1: Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.5)

Manually place libraries into a pool.

Create only one pool per step.

Step input: NTP (normalized libraries)

Step output: Bulk pool

Validate Unique Indexes automation is not used. This functionality is handled by the core Clarity LIMS configuration for pooling.

Validate Inputs Flowcell Type and Single Pool Automation

Automatically triggered on exit of the Pooling screen, this automation completes the following actions:

- Checks that all samples in the pool have the same Flowcell Type assigned to them.
- Checks that only one pool has been created.
- Changes the value of error and logging messages to reference the type of pool passed as 'bulk' pool.

```
/opt/gls/clarity/bin/java \
-jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar \
script:validate_flowcell_for_input_pools \
-i {stepURI:v2} \
-u {username} \
-p {password} \
-l {compoundOutputFileLuid1} \
-validateSingleOutput true \
-poolType bulk"
```

Calculate Volumes Automation

Triggered when the Calculate Volumes button is selected on the Record Details screen, this automation completes the following actions:

- Calculates the number of samples in the pool.

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: + 1
```

- Sets the value of the Bulk Pool Volume (µl) and PhiX Volume (µl) field, based on the selected Flowcell Type.

```
step.::PhiX Volume (ul):: = !step.hasValue(::% PhiX (0.25nM) Spike-In::) ? 0 :
step.::% PhiX (0.25nM) Spike-In::;
if (input.::Flowcell Type:: == ::SP::) {
step.::Bulk Pool Volume (ul):: = step.'Number of Lanes to Sequence' * 18 + 30;
step.::PhiX Volume (ul):: *= 1.9;
};
if (input.::Flowcell Type:: == ::S1::) {
step.::Bulk Pool Volume (ul):: = step.'Number of Lanes to Sequence' * 18 + 30;
step.::PhiX Volume (ul):: *= 1.9;
};
if (input.::Flowcell Type:: == ::S2::) {
step.::Bulk Pool Volume (ul):: = step.'Number of Lanes to Sequence' * 22 + 30;
step.::PhiX Volume (ul):: *= 1.9;
};
```

```
if (input.::Flowcell Type:: == ::S4::) {
step.::Bulk Pool Volume (ul):: = step.'Number of Lanes to Sequence' * 30 + 30;
step.::PhiX Volume (ul):: *= 2.2;
};
if (step.::PhiX Volume (ul):: == 0) {
step.::PhiX Volume (ul):: = ::::;
};
```

- Calculates the Per Sample Volume (µl) to be added to the pool.

```
input.::Per Sample Volume (µl)::= (((input.::Final Loading Concentration (pM)::*
5/ 1000) / input.::Normalized Molarity (nM)::) * step.::Bulk Pool Volume (µl)::)
/ step.::Number of Samples in Pool::
```

> To ensure accurate pipetting of each sample in a pool for sequencing, the Per Sample Volume (µl) value must be equal to or higher than the Minimum Per Sample Volume (µl) (default value set at 5, may be edited).
>
> Assuming the default Minimum Per Sample Volume (µl) value of 5, completes the following steps for a given batch:
>
> 1. If the smallest Per Sample Volume (µl) value is less than 5, automatically assigns a value of 5 to the sample Adjusted Per Sample Volume (µl) field.
> 2. Adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

- Calculates the Total Sample Volume (µl) field value.

```
step.::Total Sample Volume (µl):: = step.::Total Sample Volume (µl):: +
input.::Adjusted Per Sample Volume (µl)::
```

- If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) field value.

```
if (step.::Total Sample Volume (µl):: >= step.::Bulk Pool Volume (µl)::)
{output.::RSB Volume (µl):: = 0} else {output.::RSB Volume (µl):: = step.::Bulk
Pool Volume (µl):: - step.::Total Sample Volume (µl)::} ;
```

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs.

```
output.::Flowcell Type::= input.::Flowcell Type::;output.::Loading Workflow
Type::= input.::Loading Workflow Type::;
```

- Uses the `NovaSeq_Xp_Bulk_Pool.csv` and `NovaSeq_Xp_Bulk_Pool2.csv` template files to generate a single CSV file containing information about the bulk pool and the samples it contains. The generated file is available for download on the Step Setup screen of the Dilute, Denature, and ExAmp (NovaSeq 6000 v3.5) step.

```
&& /opt/gls/clarity/bin/java \-jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar \
```

```
  -i {stepURI:v2} \
  -u {username} \
  -p {password} \
  -l {compoundOutputFileLuid1} \ script:driver_file_generator \
  -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_Bulk_
Pool.csv \
  -o {compoundOutputFileLuid0}.csv \ script:driver_file_generator \
  -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_Bulk_
Pool2.csv \
  -o append.csv \
&& cat append.csv >> {compoundOutputFileLuid0}.csv"
```

- Resets the Total Sample Volume (µl) and Number of Samples in Pool field values so that the automation is idempotent.

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: - 1;
step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: -
input.::Adjusted Per Sample Volume (ul)::
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation completes the following actions:

- Copies the Flowcell Type values from the step inputs to the step outputs.
- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol.

```
output.::Flowcell Type:: = input.::Flowcell Type:: ; nextStep = ::ADVANCE::
```

Master Step Fields

The following table provides field configuration details fields defined on the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.5) step.

Table 146 Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.5) Master Step Field Configuration

Field Name	Field Type	Options	Additional Additional Options and Dropdown Items
% PhiX (0.25nM) Spike-in	Numeric		Range = 0–100

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Field Name	Field Type	Options	Additional Additional Options and Dropdown Items
Bulk Pool Volume (µl) (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 2
Minimum Per Sample Volume (uL)	Numeric	Required Field	Decimal places displayed = 2 Default • 5
Number of Lanes to Sequence	Numeric	Required Field	Decimal places displayed = 0
Number of Samples in Pool (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 0 Default • 0
PhiX Volume (µl) (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 2
Total Sample Volume (µl) (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 0 Default • 0

Global Fields

The following table lists the global custom fields that are configured to display on the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.5) step.

Table 147 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			

Field Name	Field Type	Options	Additional Options and Dropdown Items
RSB Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Flowcell Type	Text Dropdown	Required Field	Presets • SP • S1 • S2 • S4
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User Interaction

Refer to Run Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.5) step section in *NovaSeq 6000 Integration v3.5.0 Validation and Troubleshooting* on page 514.

Step 2: Dilute, Denature and ExAmp (NovaSeq 6000 v3.5)

In this step, pooled samples are denatured and diluted by the addition of DPX, NaOH, Tris-HCl, and RSB. Manually create working pools based on the number of lanes you want to sequence.

Step input: Bulk pool

Step output: Working pool - variable number, choose how many working pools to create per bulk pool

Validate Inputs Flowcell Type Automation

Automatically triggered at the beginning of the step, this automation invokes the `validate_flowcell_for_input_pools` script. This script completes the following actions:

- Checks the inputs to the step and validates that the Flowcell Type field is set to a valid value (SP, S1, S2, or S4) and that all inputs have the same value for this field.
- The container type selected matches the value in the Flowcell Type field.

```
bash -l -c "/opt/gls/clarity/bin/java \
-jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar \
script:validate_flowcell_for_input_pools \
-i {stepURI:v2} \
-u {username} \
-p {password} \
```

```
-l {compoundOutputFileLuid1}"
```

Calculate Volumes Automation

Automatically triggered on entry to of the Record Details screen, this automation sets the value of the following fields based on the Flowcell Type:

- BP Aliquot Volume (µl)
- NaOH Volume (µl)
- Tris-HCl Volume (µl)
- DPX1 Volume (µl)
- DPX2 Volume (µl)
- DPX3 Volume (µl)
- Mastermix per Lane (µl)

```
if ( input.::Flowcell Type:: == ::SP:: ) {
output.::BP Aliquot Volume (ul):: = 18 ;
output.::NaOH Volume (ul):: = 4.50 ;
output.::Tris-HCl Volume (ul):: = 4.50 ;
step.::DPX1 Volume (ul):: = 126 ;
step.::DPX2 Volume (ul):: = 18 ;
step.::DPX3 Volume (ul):: = 66 ;
output.::Mastermix per Lane (ul):: = 63
};
if (input.::Flowcell Type:: == ::S1::) {
output.::BP Aliquot Volume (ul):: = 18;
output.::NaOH Volume (ul):: = 4.50;
output.::Tris-HCl Volume (ul):: = 4.50;
step.::DPX1 Volume (ul):: = 126;
step.::DPX2 Volume (ul):: = 18;
step.::DPX3 Volume (ul):: = 66;
output.::Mastermix per Lane (ul):: = 63
};
if (input.::Flowcell Type:: == ::S2::) {
output.::BP Aliquot Volume (ul):: = 22;
output.::NaOH Volume (ul):: = 5.50;
output.::Tris-HCl Volume (ul):: = 5.50;
step.::DPX1 Volume (ul):: = 126;
step.::DPX2 Volume (ul):: = 18;
step.::DPX3 Volume (ul):: = 66;
```


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```
output.::Mastermix per Lane (ul):: = 77
};
if (input.::Flowcell Type:: == ::S4::) {
output.::BP Aliquot Volume (ul):: = 30;
output.::NaOH Volume (ul):: = 7.50;
output.::Tris-HCl Volume (ul):: = 7.50;
step.::DPX1 Volume (ul):: = 315;
step.::DPX2 Volume (ul):: = 45;
step.::DPX3 Volume (ul):: = 165;
output.::Mastermix per Lane (ul):: = 105
};
```

The automation also completes the following actions:

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs.

```
output.::Flowcell Type::= input.::Flowcell Type::;
output.::Loading Workflow Type::= input.::Loading Workflow Type::;
```

- Uses the `NovaSeq_Xp_Working_Pool.csv` and `NovaSeq_Xp_Working_Pool2.csv` template files to generate a single CSV file containing information about the DPX volume and the volume of BP Aliquot, Mastermix, NaOH, and Tris-HCl to add per working pool. The generated file is available for download on the Step Setup screen of the Load to Flowcell (NovaSeq 6000 v3.5) step.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_Working_
Pool.csv \
-o {compoundOutputFileLuid0}.csv \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_Working_
Pool2.csv \
-o append.csv \
&& cat append.csv >> {compoundOutputFileLuid0}.csv"
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation:

- Copies the Flowcell Type values from the step inputs to the step outputs.
- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ; nextStep = ::ADVANCE::
```

Master Step Fields

The following table lists field configuration details defined on the Dilute, Denature, and ExAmp (NovaSeq 6000 v3.5) step. These field values are set by a script and are not editable while running the step.

Table 148 Dilute, Denature, and ExAmp (NovaSeq v3.5) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
DPX1 Volume (µl)	Numeric	Read Only	Decimal places displayed = 0
DPX2 Volume (µl)	Numeric	Read Only	Decimal places displayed = 0
DPX3 Volume (µl)	Numeric	Read Only	Decimal places displayed = 0

Global Fields

The following table lists the global custom fields that are configured to display on the Dilute, Denature, and ExAmp (NovaSeq 6000 v3.5) step.

Table 149 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
BP Aliquot Volume (µl)	Numeric	Read Only	Decimal places displayed = 0
Flowcell Type	Text Dropdown	Required Field	Presets • SP • S1 • S2 • S4
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

Field Name	Field Type	Options	Additional Options and Dropdown Items
Mastermix per Lane (µl)	Numeric	Read Only	Decimal places displayed = 0
NaOH Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Tris-HCl Volume (µl)	Numeric	Read Only	Decimal places displayed = 2

User Interaction

Refer to Run Dilute, Denature & ExAmp (NovaSeq 6000 v3.5) step section in *NovaSeq 6000 Integration v3.5.0 Validation and Troubleshooting* on page 514.

Step 3: Load to Flowcell (NovaSeq 6000 v3.5)

In this step, scan the flow cell barcode into the Clarity LIMS and manually place the working pools into the lanes of the flow cell for the NovaSeq run. This step validates the run setup information and generates the sample sheet file.

Step input: Working pool

Step output: Flow cell (output containers: SP, S1, and S2 with 2 lanes, and S4 with 4 lanes)

Validate Inputs and Selected Container Automation

Automatically triggered at the beginning of the step, this automation invokes the `validate_flowcell_for_input_pools` and `validateSelectedContainer` scripts. These scripts validate the step inputs and the container selected by the user, as follows.

- Checks that the Flowcell Type field has been set to a valid value (SP, S1, S2, or S4) and that all inputs have the same value.
- Checks that the number of outputs matches the number of lanes on the selected flow cell type. If validation fails, an error message informs that the number of working pools does not match the number of lanes available on the flow cell.
  The following table defines the number of samples allowed:

Flow Cell Type	Number of Working Pools
SP	2
S1	2
S2	2
S4	4

- Checks that the container type selected on entry to the Placement screen matches the value in the Flowcell Type field. If validation fails, an error message displays.

```
bash -l -c "/opt/gls/clarity/bin/java \
-jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar
\script:validate_flowcell_for_input_pools \
  -i {stepURI:v2} \
  -u {username} \
  -p {password} \
  -l {compoundOutputFileLuid1} \
  -validateSelectedContainer true"
```

Validate Flowcell Barcode Automation

Automatically triggered on exit of the Placement screen, this automation validates the flow cell barcode scanned into the Clarity LIMS by the user, using the following logic:

if (input.::Flowcell Type:: == ::SP:: && !output.container.name.matches(::[A-Z0-9]{5}DRX[XY2357]::)) {

fail (::Invalid Flowcell Barcode. Please verify and try again.::)

};

if (input.::Flowcell Type:: == ::S1:: && !output.container.name.matches(::[A-Z0-9]{5}DRX[XY2357]::)) {

fail (::Invalid Flowcell Barcode. Please verify and try again.::)

};

if (input.::Flowcell Type:: == ::S2:: && !output.container.name.matches(::[A-Z0-9]{5}DMX[XY2357]::)) {

fail (::Invalid Flowcell Barcode. Please verify and try again.::)

};

if (input.::Flowcell Type:: == ::S4:: && !output.container.name.matches(::[A-Z0-9]{5}DSX[XY2357]::)) {

fail (::Invalid Flowcell Barcode. Please verify and try again.::)

};

- Copies the Flowcell Type and Loading Workflow Type field values from step inputs to outputs:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ;
output.::Loading Workflow Type:: = input.::Loading Workflow Type::
```

Validate Run Setup and Generate Sample Sheet Automation

The script definition portion of Validate Run Setup has been moved into the Validation Script custom field due to the 4000 character limitation.

- If modification is required to the Validate Run Setup script, perform the changes directly on Validation Script custom field default value.

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- The script definition portion of Generate Sample Sheet is unchanged.

Automatically triggered by selecting a button on the Record Details screen, this automation:

- Copies the Flowcell Type from the step input to the Run Mode field (hidden):

```
step.::Run Mode:: = input.::Flowcell Type::
```

- Validates the parameters entered on the Record Details screen, which are used to set up the run and generate the sample sheet file. For more information, refer to the *Respond to Validation / Recipe Request Call from Instrument* section of *NovaSeq 6000 Run* on page 501.
  - Experiment Name may only contain alphanumeric, dash, or underscore characters. Spaces are not permitted.
  - When Workflow Type = No Index, both Index Read 1 and Index Read 2 must be zero.
  - When Workflow Type = Single Index, Index Read 1 must be greater than zero and Index Read 2 must be zero.
  - When Workflow Type = Dual Index, both Index Read 1 and Index Read 2 must be greater than zero.

```
if(!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) {
fail(::Experiment Name contains prohibited characters. Allowed characters
are: a-z, A-Z, 0-9, -, and _::)
};
if (step.::Workflow Type:: == ::No Index::) {
if (step.::Index Read 1:: != 0 || step.::Index Read 2:: != 0) {
fail(::Index Read 1 and Index Read 2 must be 0 if No Index is selected.::)
}
} else if (step.::Workflow Type:: == ::Single Index::) {
if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) {
fail(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if
Single Index is selected.::)
}
} else {
if (step.::Index Read 1:: == 0 || step.::Index Read 2:: == 0) {
fail(::Index Read 1 and Index Read 2 must be greater than 0 if Dual Index is
selected.::)
}
};
```

- Checks the Paired End and Read 2 Cycles field values.
  - If Paired End = True and Read 2 Cycles value is 0, an error displays.
  - If Paired End = False, Read 2 Cycles must be zero and UMI - Read 2 Length and UMI - Read 2 Start From Cycle must be empty.

```
if (step.::Paired End::.toBoolean()) {
```


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```
if (step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) {
fail(::Read 1 Cycles and Read 2 Cycles must be greater than 0 if Paired End
is True.::)
}
} else {
if (step.::Read 2 Cycles:: != 0) {
fail(::Read 2 Cycles must be 0 if Paired End is False.::)
};
if (step.hasValue(::UMI - Read 2 Length::) || step.hasValue(::UMI - Read 2
Start From Cycle::)) {
fail(::UMI - Read 2 Length and UMI - Read 2 Start From Cycle cannot be
defined if Paired End is False.::)
}
};
```

- Checks the Flowcell field value.
  If Flowcell Type value is not SP, checks that the value of both Read 1 Cycles and Read 2 Cycles is 151 or less. If value is greater than 151, generates an error message.

```
if (input.::Flowcell Type::!=::SP:: && step.::Read 1 Cycles:: > 151) {
fail(::Read 1 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
}
if (input.::Flowcell Type::!=::SP:: && step.::Read 2 Cycles:: > 151) {
fail(::Read 2 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
}
```

- Validates UMI Settings:
  - If UMI - Read 1 Length has value, UMI - Read 1 Start From Cycle must have value.
  - If UMI - Read 1 Length has no value, UMI - Read 1 Start From Cycle must not have value.
  - If UMI - Read 2 Length has value, UMI - Read 2 Start From Cycle must have value.
  - If UMI - Read 2 Length has no value, UMI - Read 2 Start From Cycle must not have value.
  - If UMI - Read 1 Length has not value, UMI - Read 2 Length must not have value.
  - If UMI - Read 1 Start From Cycle has no value, UMI - Read 2 Start From Cycle must not have value.

```
if (step.hasValue(::UMI - Read 1 Length::) && !step.hasValue(::UMI - Read 1
Start From Cycle::)) {
fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 1
Length is greater than 0.::)
};
if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 1
Start From Cycle::)) {
```


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```
fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 1 Start From
Cycle is greater than 0.::)
};
if (step.hasValue(::UMI - Read 2 Length::) && !step.hasValue(::UMI - Read 2
Start From Cycle::)) {
fail(::UMI - Read 2 Start From Cycle must be greater than 0 if UMI - Read 2
Length is greater than 0.::)
};
if (!step.hasValue(::UMI - Read 2 Length::) && step.hasValue(::UMI - Read 2
Start From Cycle::)) {
fail(::UMI - Read 2 Length must be greater than 0 if UMI - Read 2 Start From
Cycle is greater than 0.::)
};
if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 2
Length::)) {
fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 2 Length is
greater than 0.::)
};
if (!step.hasValue(::UMI - Read 1 Start From Cycle::) && step.hasValue(::UMI
- Read 2 Start From Cycle::)) {
fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 2
Start From Cycle is greater than 0.::)
};
```

- Validates Analysis Software Version to make sure it is a valid version string.

```
if (step.hasValue(::Analysis Software Version::) && !step.::Analysis Software
Version::.matches(::^(\\d+)(\\.\\d+)*$::)) {
fail(::Analysis Software Version contains prohibited characters. Allowed
characters are 0-9 and period. It shall start and end with numbers and separated
by single period e.g. 3.8.4::)
};
```

- Checks Override Cycles field value. This value can contain only Y, N, I, U, 0–9, and semicolon characters.

```
if (step.hasValue(::Override Cycles::) && !step.::Override Cycles::.matches(::
[YNIU0-9;]+::)) {
fail(::Override Cycles contains prohibited characters. Allowed characters are:
Y, N, I, U, 0-9 and ;. Example: N1Y150;I8;I7N1;Y141U10.::)
};
```

- Outputs the Settings Header in sample sheet when UMI - Read 1 Length or UMI - Read 2 Length have values.

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```
step.::Settings Header:: = step.hasValue(::UMI - Read 1 Length::) ||
step.hasValue(::UMI - Read 2 Length::) ? ::[Settings]:: : ::::;
```

- Sets the next step for samples to ADVANCE, which advances to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5) step.

```
nextStep = ::ADVANCE::
```

- Generates the sample sheet and attaches it to the step. The sample sheet template depends on the selected value for Samplesheet Format and/or Reverse Complete Workflow. For more information, refer to *Sample Sheet Generation* on page 500.

```
&& if [ '{udf:Samplesheet Format}' == 'V2' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Samplesheet_v2.csv'; \
elif [ '{udf:Reverse Complement Workflow}' == 'true' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Reverse_Complement_Samplesheet.csv'; \
elif [ '{udf:Reverse Complement Workflow}' == 'false' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Samplesheet.csv'; \
else echo 'UDF Reverse Complement Workflow is empty or undefined.'; exit -1; fi
\
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/$templateFilename \
-o {compoundOutputFileLuid0}.csv \
-q true \
-destLIMSID {compoundOutputFileLuid0} \
-l {compoundOutputFileLuid1}"
```

Master Step Fields

There are 27 fields defined on the Load to Flowcell (NovaSeq 6000 v3.5) step.

The following table lists the field configuration details.

Table 150 Load to Flowcell (NovaSeq 6000 v3.5) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Analysis Software Version	Text		Only applicable for the V2 sample sheet. Otherwise, leave this field blank.

Field Name	Field Type	Options	Additional Options and Dropdown Items
BaseSpace Sequence Hub Configuration	Text Dropdown	Required Field	Presets • Not Used • Run Monitoring Only • Run Monitoring and Storage
Custom Recipe Path	Text		
Experiment Name	Text	Required Field	
Index Read 1	Numeric Dropdown	Required Field Custom Entries	Range = 0–20 Decimal places displayed = 0 Presets • 0 • 6 • 8
Index Read 2	Numeric Dropdown	Required Field Custom Entries	Range = 0–20 Decimal places displayed = 0 Presets • 0 • 6 • 8
Library Tube Barcode	Text	Required Field	
Output Folder	Text	Required Field	
Override Cycles	Text		Only applicable for the V2 sample sheet. Otherwise, leave this field blank.
Paired End	Text Dropdown	Required Field	Presets • True • False

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Field Name	Field Type	Options	Additional Options and Dropdown Items
Read 1 Cycles	Numeric Dropdown	Required Field Custom Entries	Range = 1–251 Decimal places displayed = 0 Presets • 251* • 151 • 101 • 51 *The value of 251 is only supported for SP flow cell type. For all other flow cell types, the maximum value is 151.
Read 2 Cycles	Numeric Dropdown	Required Field Custom Entries	Range = 0–251 Decimal places displayed = 0 Presets • 251* • 151 • 101 • 51 *The value of 251 is only supported for SP flow cell type. For all other flow cell types, the maximum value is 151.
Reverse Complement Workflow	Toggle Switch	Required Field	Default • Yes
Run Mode (not displayed in user interface)	Text Dropdown	Read Only	Presets • SP • S1 • S2 • S4
Samplesheet Format	Text Dropdown	Required Field	Presets • V1 (default) • V2
Settings Header (not displayed in user interface)	Text	Read Only	

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Field Name	Field Type	Options	Additional Options and Dropdown Items
UMI - Read 1 Length	Numeric		Range = 1
UMI - Read 1 Start From Cycle	Numeric		Range = 1
UMI - Read 2 Length	Numeric		Range = 1
UMI - Read 2 Start From Cycle	Numeric		Range = 1
Use Custom Index Read 1 Primer	Toggle Switch		Default • None Set
Use Custom Read 1 Primer	Toggle Switch		Default • None Set
Use Custom Read 2 Primer	Toggle Switch		Default • None Set
Use Custom Recipe	Toggle Switch	Required Field	Default • No
Validation Script	Multiline Text	Required Field Read Only	Default value provided in the *Validation Script* on page 496 section following the table. Do not remove this field as it is used by Validate Run Setup and Generate Sample Sheet automation script.
Workflow	Text	Read Only	Default • GenerateFASTQ
Workflow Type	Text Dropdown	Required Field	Presets • No Index • Single Index • Dual Index • Custom

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Validation Script

step.::Run Mode:: = input.::Flowcell Type::; if (!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) { fail(::Experiment Name contains prohibited characters. Allowed characters are: a-z, A-Z, 0-9, -, and _::) }; if (step.::Workflow Type:: == ::No Index::) { if (step.::Index Read 1:: != 0 || step.::Index Read 2:: != 0) { fail(::Index Read 1 and Index Read 2 must be 0 if No Index is selected.::) } } else if (step.::Workflow Type:: == ::Single Index::) { if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) { fail(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if Single Index is selected.::) } } else { if (step.::Index Read 1:: == 0 || step.::Index Read 2:: == 0) { fail(::Index Read 1 and Index Read 2 must be greater than 0 if Dual Index is selected.::) } }; if (step.::Paired End::.toBoolean()) { if (step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) { fail(::Read 1 Cycles and Read 2 Cycles must be greater than 0 if Paired End is True.::) } } else { if (step.::Read 2 Cycles:: != 0) { fail(::Read 2 Cycles must be 0 if Paired End is False.::) }; if (step.hasValue(::UMI - Read 2 Length::) || step.hasValue(::UMI - Read 2 Start From Cycle::)) { fail(::UMI - Read 2 Length and UMI - Read 2 Start From Cycle cannot be defined if Paired End is False.::) } }; if (input.::Flowcell Type:: != ::SP:: && step.::Read 1 Cycles:: > 151) { fail(::Read 1 Cycles must not be larger than 151 if it is not SPrime Flowcell::) }; if (input.::Flowcell Type:: != ::SP:: && step.::Read 2 Cycles:: > 151) { fail(::Read 2 Cycles must not be larger than 151 if it is not SPrime Flowcell::) }; if (step.hasValue(::UMI - Read 1 Length::) && !step.hasValue(::UMI - Read 1 Start From Cycle::)) { fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 1 Length is greater than 0.::) }; if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 1 Start From Cycle::)) { fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 1 Start From Cycle is greater than 0.::) }; if (step.hasValue(::UMI - Read 2 Length::) && !step.hasValue(::UMI - Read 2 Start From Cycle::)) { fail (::UMI - Read 2 Start From Cycle must be greater than 0 if UMI - Read 2 Length is greater than 0.::) }; if (!step.hasValue(::UMI - Read 2 Length::) && step.hasValue(::UMI - Read 2 Start From Cycle::)) { fail (::UMI - Read 2 Length must be greater than 0 if UMI - Read 2 Start From Cycle is greater than 0.::) }; if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 2 Length::)) { fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 2 Length is greater than 0.::) }; if (!step.hasValue(::UMI - Read 1 Start From Cycle::) && step.hasValue(::UMI - Read 2 Start From Cycle::)) { fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 2 Start From Cycle is greater than 0.::) }; if (step.hasValue(::Analysis Software Version::) && !step.::Analysis Software Version::.matches(::^(\\d+)(\\.\\d+)*$::)) { fail(::Analysis Software Version contains prohibited characters. Allowed characters are 0-9 and period. It shall start and end with numbers and separated by single period e.g. 3.8.4::) }; if (step.hasValue(::Override Cycles::) && !step.::Override Cycles::.matches(::[YNIU0-9;]+::)) { fail (::Override Cycles contains prohibited characters. Allowed characters are: Y, N, I, U, 0-9 and ;. Example: N1Y150;I8;I7N1;Y141U10.::) }; step.::Settings Header:: = step.hasValue(::UMI - Read 1 Length::) || step.hasValue(::UMI - Read 2 Length::) ? ::[Settings]:: : ::::; nextStep = ::ADVANCE::

Global Fields

The following table lists the global custom fields that are configured to display on the Load to Flowcell (NovaSeq 6000 v3.5) step.

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Table 151 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Analyte in Derived Sample			
Flowcell Type	Text Dropdown	Required Field	Presets • SP • S1 • S2 • S4
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User Interaction

Refer to Run Load to Flowcell (NovaSeq 6000 v3.5) step section in *NovaSeq 6000 Integration v3.5.0 Validation and Troubleshooting* on page 514.

Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5)

This final protocol contains one fully automated step, AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5), described in the following section.

This is a fully automated step. Do not add samples to the Ice Bucket or start the step manually. The sequencing service does not update samples correctly if they have been manually started.

Step 1: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5)

Step input: Library tube from NovaSeq Standard or flow cell from NovaSeq Xp protocol

Step output: Result file/measurement

In this step, pooled samples are sequenced on the NovaSeq instrument and the run metrics are recorded in Clarity LIMS.

Set Next Steps Automation

By default, the Set Next Steps automation is disabled (set to Not Used) and the Sequencer API sets the next step for samples.

If necessary, override the default next step behavior used by the Sequencer API by performing the following steps:

1. Enable the automation and use it to set custom next step behavior and generate a log file. By default, the automation sets the next step to ADVANCE and the samples complete the protocol.

2. Configure the automation to trigger automatically on exit of the Record Details screen.
3. Disable or configure auto-complete behavior by setting the values of the run.autoComplete and autoCompleteOnlyAtSuccess properties. For details, refer to *Configuring autoComplete Properties* on page 512.

Update Lane Number Automation

This automation triggers automatically upon entry to the Record Details screen.

Do not disable or modify this automation to ensure the Lane Number displays properly.

Master Step Fields

The following fields are configured on the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5) step in Clarity LIMS:

- Current Cycle
- Current Read
- Firmware Version
- Flow Cell Expiration Date
- Flow Cell ID
- Flow Cell Lot Number
- Flow Cell Mode
- Flow Cell Part Number
- Flow Cell Side
- Instrument Control Software Version
- Instrument ID
- Instrument Type
- Lane Counter
- Loading Workflow Type
- Output Folder
- RTA Version
- Run Completion Date
- Run ID
- Run Status
- Sequencing Log

Global Fields

The following fields are used to capture the run metrics in Clarity LIMS:

- % Aligned R1
- % Aligned R2
- % Bases >=Q30 R1
- % Bases >=Q30 R2
- % Error Rate R1
- % Error Rate R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- %PF R1
- %PF R2
- Cluster Density (K/mm^2) R1
- Cluster Density (K/mm^2) R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- Reads PF (M) R1
- Reads PF (M) R2
- Yield PF (Gb) R1
- Yield PF (Gb) R2

Note the following details:

- Values are aggregated across all lanes. Some values (eg, Yield PF (Gb) R1) are summed while others are averaged.
- The names listed previously are the default global custom field names installed with the NovaSeq Integration v3.5.0 configuration provided in the Illumina Preset Protocols (IPP) v2.4 or later.
- All global configuration fields are configured on the Container entity.
- All field names are configurable through the Custom Fields screen in the Global Fields tab.

- If field names are changed in Clarity LIMS, they must also be changed through the `run.metricUdfNames` properties file in the `application.yml` file for the Sequencer API. For more information, refer to the *application.yml Properties* section in *Components Installed* on page 506. All fields are configured to be visible in the Sample Details table on the Record Details screen for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5) step.

These global custom fields are configured on the Container entity, visible on each input, but they should not be confused with per-lane metrics.

All run metrics tracked in Clarity LIMS are overall metrics for the run. There are currently no per-lane metrics provided by NovaSeq Control Software.

Sample Sheet Generation

The sample sheet is generated on the step before the run, which is Dilute and Denature (NovaSeq 6000 v3.5) in the NovaSeq Standard protocol or Load to Flowcell (NovaSeq 6000 v3.5) in the NovaSeq Xp protocol. This step places samples on the library tube or flow cell that are loaded in the NovaSeq 6000 instrument.

In the default configuration, the Validate Run Setup and Generate Sample Sheet automation generates one CSV format sample sheet file for use with bcl2fastq v2.20 downstream analysis.

The sample sheet is uploaded to the NVCS via the `/Illumina/Sequencer/v2/sequencing-run/files` endpoint to the Sequencer API. The file endpoint allows for a file to be downloaded from the Clarity LIMS using OAuth (instead of Basic Authentication), which is required for the NovaSeq 6000 instrument.

The run recipe response sets the sample sheet URL to the link to download the file from this endpoint and sets the sampleSheetRequiresOAuth value to true.

Sample Sheet Content

The sample sheet content is controlled by the following master step fields that display in the Step Details area of the Record Details screen.

- Experiment Name
- Read 1 Cycles
- Read 2 Cycles
- Workflow
- Reverse Complement Workflow
- Samplesheet Format

Depending on Samplesheet Format field value selection, a v1 or v2 sample sheet is generated. For detailed information on the following items, refer to the *Bcl2fastq2 Sample Sheet Generation* section of *Illumina Instrument Sample Sheets (NGS v5.17 and later)*:

- Sample sheet generation script parameters and usage
- Sample sheet data and configuration options
- Enabling unique FASTQ file names per sequencing run

NovaSeq 6000 Run

The following steps outline the sequence of events that occurs when a flow cell is loaded onto the NovaSeq 6000 instrument.

User Authentication and Login

User authentication and login to the NovaSeq instrument is achieved via the getSequencerLoginURL endpoint (found at `/Illumina/Sequencer/v2/sequencing-run/login`). This endpoint returns the URL that the instrument uses to get an access token.

1. The Sequencer API service provides the NovaSeq Control Software (NVCS) with a URL for the Clarity LIMS login screen and the NVCS displays this screen to the user. Log in to automatically authorize the NVCS to use your account to access the Clarity LIMS.
2. Enter credentials:
   - Username and password are passed to the Clarity LIMS server to make sure that the access is valid.
   - By default, the Sequencer API service tries to contact the Clarity LIMS at `http://localhost:9080/clarity/`. This URL is set by the installation script, but it can be configured with the clarity.url property in the `application.yml` file. For more information, refer to the *application.yml Properties* section of *Components Installed* on page 506.
3. The user is redirected to the Sequencer login page at `/Illumina/Sequencer/v2/sequencer_login`.
   - If authentication is successful, the screen displays a success message. The Clarity LIMS server provides the OAuth token and username by returning them in redirect to `/Illumina/Sequencer/v2/sequencer_login/Success`. This redirect does not reload the page, but allows the NovaSeq to continue with run setup.
   - If authentication fails, the user is left on the login screen and a warning message informs them of the failure. A background redirect to `/Illumina/Sequencer/v2/sequencer_login/Error` informs the NovaSeq 6000 of the failure.

Respond to Validation / Recipe Request Call from Instrument

1. The `sequencing-run/recipe/novaseq` endpoint consumes a POST request with a LibraryContainerI, FlowCellId, and reagents information.
   - LibraryContainerId, FlowCellId, and reagent information is provided by the NovaSeq 6000.

- LibraryContainerId and FlowCellId should match either the library tube ID or flow cell ID of the corresponding containers in Clarity LIMS.
- If there are no containers or multiple containers, an error is generated.

2. All populated containers whose name matches either the LibraryContainerId or FlowCellId are validated as follows.
   - All samples in the container must be queued for a sequencing run step.
   - All samples must be queued for only one sequencing run step.
   - All samples must be queued for the same sequencing run step.

   Configure the steps that are considered sequencing run steps in the novaseq.sequenceStepNames property in the `application.yml` file. The property is a list and each list entry must be the exact name of a sequencing step, not the name of the underlying master step/process type. For more information, refer to the *application.yml Properties* section of *Components Installed* on page 506.

3. After a single container has passed validation, the run setup information (run recipe request) is returned to the NVCS via the API and the run recipe is generated.
   - The run recipe information is read from fields configured on the parent process/master step of the samples in the container. The workflow must include recipe definition in that step.
   - By default, the parent process/master step is the Dilute and Denature (NovaSeq 6000 v3.5) or Load to Flowcell (NovaSeq 6000 v3.5) step, depending on workflow.
   - The default field values match the names of the fields in the NovaSeq v3.5.0 configuration included in IPP. The field names are configurable through the recipe.udfNames.* properties. Each of these properties corresponds to an entry in the recipe and the value of a property defines the name of the field that is used to populate that entry in the recipe.
   - It is assumed that this parent process/master step removes any illegal characters from the Experiment Name field.
   - The Clarity LIMS download link to the sample sheet created on the run setup step is used for the SampleSheet value in the recipe. The name of the output of the sample sheet on the setup step can be configured through the recipe.sampleSheet.outputName property (Sample Sheet by default) in the `application.yml` file. For more information, refer to the *application.yml Properties* section of *Components Installed* on page 506. If no matching output is found, or no file was attached to the output, then the value of the recipe.sampleSheet.notAvailableValue property (Not Available by default) is used in the recipe.

4. After the recipe is generated, it is returned as a *.json file in the response, in the form defined by the Swagger definition for the API.

Run Recipe Content

The content of the run recipe is controlled by the sample sheet content and the fields configured on the Dilute and Denature (NovaSeq 6000 v3.5) and Load to Flowcell (NovaSeq 6000 v3.5) steps:

The following example shows the run recipe content:

```
{
"run_name": "NVCS16027_SPrime_2x250",
"run_mode": "S4",
"workflow_type": "DualIndex",
"librarytube_id": "NV0025867-LIB",
"flowcell_id": "",
"sample_loading_type": "NovaSeqStandard",
"rehyb": false,
"paired_end": true,
"read1": 151,
"read2": 151,
"index_read1": 8,
"index_read2": 8,
"output_folder": "\\\\network_path\\run_data",
"samplesheet": "<path to sample sheet on LIMS server>",
"require_samplesheet_authentication": true,
"usecustomrecipe": false,
"customRecipe": null,
"use_basespace": false,
"basespace_mode": null,
"use_custom_read1_primer": false,
"use_custom_read2_primer": false,
"use_custom_index_read1_primer": false
}
```

Respond to Sequencing Started Call from Instrument

1. Matching container for the run (library tube or flow cell) is found, and contents are validated to be queued for the correct step.

   If no matching container is found or if the samples in the container are incorrectly queued, an error is thrown and the step is not started. This validation is also done during the run recipe request immediately prior, so this error case should not be encountered.

2. Reagent kits that are configured for the Sequencer API are checked against the configuration for the step to be run:

- If a kit is configured for the API but does not exist in Clarity LIMS, it is created.
- If a kit exists, but is not enabled on the step to be run, the step configuration is updated to enable it.
  - If a kit that is to be enabled is Archived, it is set to Active.
  - If a kit that is to be enabled is in Pending status, it is set to Active.
- If any kits enabled on the step are not configured for the Sequencer API, a warning is logged and the step cannot be completed by the API without user intervention.
- If any kits provided in the recipe request are not configured for the Sequencer API, they are ignored and a warning is logged during lot tracking.

3. The number of replicates for the step is determined by the type of flow cell being used in the run.
   - For Standard mode, this information is based on the number of lanes in the flow cell. This information is stored as part of the Sequencer API configuration.
   - For XP mode, there is always one replicate, because there is already one input tracked for each lane.
4. The automated sequencing step is started.
5. Reagent lots are attached to the step. The Flow Cell and Library Tube reagents in the request are ignored. Information from these reagents is tracked in Clarity LIMS as containers and custom fields. Any other reagents in the request are handled as follows.
   - If the reagent is not configured to be tracked, a warning is logged and the reagent is ignored.
   - Existing reagent lots are looked up by kit name (determined by Sequencer API configuration), name (the serial number in the request), and lot number (the lot number in the request).
   - If one or more matching reagent lots exist in Clarity LIMS, the newest one is used.
   - If the expiry date does not match the expiry in the request, or if the status is not set to Active, the lot is updated to have the correct expiry date and an Active status.

   Otherwise a new lot is created with the following configuration:
   - Reagent Kit—Matching kit as determined from Sequencer API configuration and 2.
   - Name—Serial number in request
   - Lot Number—Lot number in request
   - Expiry—Expiry date in request
   - Status—Active

   If any reagents are expected to be in the request but are not, these reagents are logged. This action prevents the step from being completed without user intervention.
6. Master step custom fields are updated with the run information parsed from the request. For more information, refer to *Respond to Record InterOps Metrics Call from Instrument* on page 505.

7. Any important events during this request are logged into the Sequencer API log file and in the Sequencing Log multiline global custom field on the step (assuming the step was started successfully). For more information, refer to *Respond to Record InterOps Metrics Call from Instrument* on page 505.

Respond to Record InterOps Metrics Call from Instrument

When the metrics endpoint receives a POST, it completes the following actions:

- Looks for a matching library tube or flow cell in Clarity LIMS.
- Find the in-progress sequencing step for that container.

When the step is found, the run metrics in the request are written to container global custom fields in Clarity LIMS.

Respond to Sequencing Completed Call from Instrument

If the sequencing run is successful, after the metrics are recorded, the API call for Sequencing Completed is received. Information is matched by Sequencing Container and the following occurs in Clarity LIMS:

1. The Current Cycle, Current Read, and Status master step custom fields are updated.
2. The multiline Sequencing Log master step field is updated.
3. A success message is recorded in the log file at the info level:

```
Sequencing run with Run ID {runId} of Step '{step.name}' ({step.limsid})
completed successfully.
```

4. The Clarity LIMS waits for any automations that are triggered automatically as it advances the step.
5. By default, the Clarity LIMS assigns the next steps on the outputs to move them on to the first configured next step. If there are no next steps, the protocol is completed.

Respond to Sequencing Errored Call from Instrument

If the sequencing run is unsuccessful, the API call for Sequencing Error is received. Information is matched by Sequencing Container and the following occurs in Clarity LIMS:

1. The Current Cycle, Current Read, and Status master step custom fields are updated.
2. The multiline Sequencing Log master step field is updated.
3. A failure message is recorded in the log file at the error level:

```
Step '${Step.name}' (${Step.limsid}) failed with Run ID ${runId}.
```

4. The step is left at the Record Details screen.

Respond to Sequencing RunEndedByUser Call from Sequencer

If the sequencing run is ended by the user, the API call for Sequencing RunEndedByUser is received. Information is matched by Sequencing Container and the following occurs in Clarity LIMS:

1. The Current Cycle, Current Read, and Status master fields are updated.
2. The multiline Sequencing Log master step field is updated.
3. The following message is recorded in the log file, at the warning level:

```
"Sequencing run with Run ID ${status.runInfo.runId} of Step
'${step.configuration.name}' (${step.limsid}) aborted by user
${status.runInfo.userName}."
```

4. The step is left at the Record Details screen.

Components Installed

The following sections describe the various components (files, properties, reagent categories / label groups, reagent kits, and containers) that are installed by default as part of this integration.

Illumina NovaSeq 6000 Integration v3.5.0 is distributed as the `BaseSpaceLIMS-sequencer-api` RPM package. This RPM package must be installed on the Clarity LIMS server.

The `BaseSpaceLIMS-sequencer-api` RPM installs the following items:

- Sequencer API WAR file
- `application.yml` configuration file
- Two configuration scripts: `configure_sequencer_api_proxy.sh` and `configure_sequencer_api_application.sh`

The NovaSeq 6000 v3 configuration is delivered through Illumina Preset Protocols (IPP) v2.4 which requires installation of the NGS Extensions Package v5.22.0 or later.

- If the NGS Extensions Package is not already installed, or if a version earlier than v5.22.0 is installed, the latest version is installed by default with the NovaSeq 6000 integration. For more information, refer to *NovaSeq 6000 Integration v3.5.0 Release Notes* on page 535.
- If NGS Extensions Package v5.22.0 or later is already installed, the upgrade is not forced or required.

NovaSeq 6000 Integration RPM Components

The following table lists the components installed by the RPM package.

Files Installed	Location and Description
`Illumina#Sequencer#v2.war`	`/opt/gls/clarity/tomcat/current/webapps` War file for NovaSeq v3.5.0 API.

Files Installed	Location and Description
`configure_sequencer_api_application.sh`	`/opt/gls/clarity/config/` Script that configures the Sequencer API application through its external `application.yml` file.
`configure_sequencer_api_env.sh`	`/opt/gls/clarity/config/` Script that configures the Clarity LIMSTomcat configuration to include Secret Util settings.
`configure_sequencer_api_proxy.sh`	`/opt/gls/clarity/config/` Script that configures the proxy to allow communication with the Sequencer API (NovaSeq v3.5.0 API) application.
`sequencer-api.conf`	`/etc/httpd/clarity/` Proxy setting to communicate with the Clarity LIMS.

application.yml Properties

The `application.yml` file is located at `opt/gls/clarity/extensions/sequencing-api/`.

- All properties are configured automatically during installation, but they can also be configured by editing the `application.yml` file and then restarting Tomcat.
- All custom field names, both for recipe and run information, are represented by properties in the file.
- For all custom fields, the names (values in this file) can be changed. However, the property names cannot be changed, removed, or added to. For example, Loading Workflow Type can be changed, but recipe.udfNames.sampleLoadingType cannot.

Table 152 application.yml Properties Installed

Property	Description
spring.profiles.active	Tells the application that it is running deployed in Tomcat. Default • tomcat (Do not change)
clarity.url	Base URL that the Sequencer API service uses to contact the Clarity LIMS. The installation script (`configure_sequencer_api_application`) prompts for this.

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Property	Description
clarity.username	Username to be used when communicating with Clarity LIMS.
security.signing-key	Private key that should be used when signing/validating OAuth tokens. Changing this invalidates any issued tokens.
security.token-expiry	Specifies (in hours) the expiry period for login tokens issued by the Sequencer API. Default • 88
novaseq.sequenceStepNames	List of NovaSeq sequencing run step names for which the integration may find samples queued. • Names must be an exact match to the name of the step in Clarity LIMS (not the master step/process type). • Each line should be indented to the same point and start with a dash (-). Default - AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5)

novaseq.flowcells	List of supported NovaSeq flow cell types.
	• Type names exactly match the values used by the NVCS run status requests.
	• To add more types, provide both type and lanes properties, indented to the same point and starting with a dash (-).
	Default
	`- type: "SP"`
	`lanes: 2`
	`- type: "S1"`
	`lanes: 2`
	`- type: "S2"`
	`lanes: 2`
	`- type: "S4"`
	`lanes: 4`

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Property	Description

novaseq.reagents	List of supported reagent kits to be tracked on sequencing steps.
	• **- lotName**—The name of the lot as it appears in the run started request.
	• - kitName—The name of the corresponding reagent kit in Clarity LIMS that the lot should be tracked as.
	• The name may differ in the NVCS request versus the Clarity LIMS configuration.
	Default
	`- lotName: "Buffer"`
	`kitName: "Buffer Cartridge"`
	`- lotName: "Cluster"`
	`kitName: "Cluster Cartridge"`
	`- lotName: "SBS"`
	`kitName: "SBS Cartridge"`

recipe.udfNames	Configures the names of the fields used in the run recipe. For more information, refer to the *Run Recipe Contents* section of *NovaSeq 6000 Run* on page 501. • Each value must be enclosed in quotes (eg, recipe.udfNames.runMode "Run Mode"). • Value provided for sampleLoadingType must match the value provided in the run configuration (eg, recipe.udfNames.sampleLoadingType "Loading Workflow Type").
recipe.sampleSheet.outputName recipe.sampleSheet.notAvailableValue	Configures the name of the sample sheet file placeholder on the step where run recipe information is populated, and the property value to use when the file is not found.

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Property	Description
run.stepUdfNames	Configures the master step field names to be used when recording run results. For more information, refer to the *Master Step Fields* section of *Step 1: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5)* in *Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5)* on page 497. • Each value must be enclosed in quotes (eg, run.stepUdfNames.runID "Run ID") • Value provided for sampleLoadingType must match the value provided in the run configuration (eg, run.stepUdfNames.sampleLoadingType "Loading Workflow Type"). It is expected that the run step field and the field on the input derived sample share a name.
run.metricUdfNames	Configures the result file custom fields/measurement global field names to be used when recording run results. These are the fields that capture the run data. For more information, refer to the *Global Fields* section of *Step 1: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5)* in *Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5)* on page 497.
run.autoComplete	Determines whether the sequencing run step should be completed automatically after parsing the run metrics on a run along with run.autoCompleteOnlyAtSuccess. For more information, refer to *Configuring autoComplete Properties* on page 512. Default • true

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Property	Description
run.autoCompleteOnlyAtSuccess	Determines which of the following options the sequencing run step should be: • Autocompleted when the run status is RunCompletedSuccessfully (if autoCompleteOnlyAtSuccess is true) • Autocompleted regardless of the run status (if autoCompleteOnlyAtSuccess is false). For more information, refer to *Configuring autoComplete Properties* on page 512. Default • true

Reagent Categories/Label Groups

- TruSeq HT Adapters v2 (D7-D5)

Reagent Kits

- Buffer Cartridge
- Cluster Cartridge
- DPX1
- DPX2
- DPX3
- NaOH
- Resuspension Buffer (RSB)
- SBS Cartridge
- Tris HCl

Container Types

- Library Tube
- SP
- S1
- S2
- S4

This integration supports the following items:

- Library tube with barcode provided in the format [A-Z]{2}[0-9]{7}-[A-Z]{3} (eg, AB1234567-XYZ)
- SP, S1, S2, or S4 flow cell with barcode provided in one of the following formats:
  - SP and S1 flow cell: [A-Z0-9]{5}DRX[XY2357]
  - S2 flow cell: [A-Z0-9]{5}DMX[XY2357]
  - S4 flow cell: [A-Z0-9]{5}DSX[XY2357]
  - Example: H1991DMXX

NVCS Configuration

For details on configuring NVCS for integration with Clarity LIMS, contact the Clarity LIMS Support team.

Routing Script Requirements

The requirements for the routing script functionality are as follows.

1. On the steps that use the routing script Define Run Format (NovaSeq 6000 v3.5) and Dilute and Denature (NovaSeq 6000 v3.5), the Next Step for all samples must be set to Remove from workflow. This value is set by a script. The value must not change in the Assign Next Steps screen.
2. In the Next Steps section of the protocol configuration screen, the method of assigning the next step must be set to Automation for the last step of the protocol.

Configuring autoComplete Properties

As of NovaSeq Integration v3.5.0, the `application.yml` configuration file contains two additional properties:

- run.autoComplete
- run.autoCompleteOnlyAtSuccess

These properties determine the conditions under which the last step of the NovaSeq 6000 workflow (AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5)) is automatically completed when the run status is "RunCompletedSuccessfully" (default behavior) or regardless of the run status.

By default, both properties are set to true and the step only completes when the run is successful. If the run fails or is aborted, manually complete the step in Clarity LIMS.

For the step to autoComplete regardless of the result of the sequencing run, change the autoCompleteOnlyAtSuccess property value to false.

Enabling/Disabling autoComplete Properties

1. Open the `application.yml` file at `/opt/gls/clarity/extensions/sequencer-api/application.yml`:

```
vi /opt/gls/clarity/extensions/sequencer-api/application.yml
```

2. Edit the value of the autoCompleteOnlyAtSuccessproperty as required.

3. Save the file.

The following table shows how the combined value of the run.autoComplete and run.autoCompleteOnlyAtSuccess properties affects the autoComplete behavior of the sequencing step.

Table 153 run.autoComplete and run.autoCompleteOnlyAtSuccess Value Matrix

run.autoComplete Property Value	run.autoCompleteOnlyAtSuccess Property Value	Outcome
true	true	• Step automatically completes only if sequencing run is successful. • If run is not successful, step does not automatically complete and run details are recorded in Clarity LIMS.
true	false	• Step automatically completes, regardless of the run status.
false	true	• Step does not automatically complete. • Run details are recorded in Clarity LIMS.
false	false	

Rules and Constraints

- The workflow configuration contains several validation checks. To make sure that the calculations work properly, it is important that you do not disable any of this validation logic. The validation checks determine the following information:
  - Which samples, and how many, can enter each step together.
  - Which samples, and how many, can be pooled together.
- The library tube ID must be unique. There should not be multiple library tube containers in the system with the same name.
- Reagent labels (indexes) must be unique.
- Only controls are permitted as unindexed samples; all other unindexed samples and pools are not permitted.
- For sample sheet generation constraints, refer to the *Bcl2fastq2 Sample Sheet Generation* section in *Illumina Instrument Sample Sheets (NGS v5.17 & later)*.

- Do not manually start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5) step. This step is a fully automated and the sequencing service does not update samples correctly if they have been manually started.
- For the automated run to start successfully, Validate Run Setup and Generate Sample Sheet must be selected.

NovaSeq 6000 Integration v3.5.0 Validation and Troubleshooting

This section explains how to validate the installation of the Illumina NovaSeq 6000 Integration Package v3.5.0.

The validation process involves the following items:

- Running samples through the Library Prep Validation workflow.
  - The workflow contains a single-step protocol that models the library prep required to produce normalized libraries. At the end of the step, the normalized libraries advance to the workflow selected.
- Running normalized libraries through the NovaSeq 6000 v3.5 workflow validates the following items:
  - Successful routing of samples from the Run Format (NovaSeq 6000 v3.5) step to the NovaSeq Standard (NovaSeq 6000 v3.5) or NovaSeq Xp (NovaSeq 6000 v3.5) step.
  - Automated generation of v1 sample sheet for use with bcl2fastq2 v2.20 analysis software or v2 sample sheet. This file automatically uploads to the sequencing system via the Sequencer API.
  - Automatic validation of run setup information. Information is uploaded to the NovaSeq Control Software (NVCS) via the Sequencer API and is used to create the run recipe and initiate the run.
  - Automated tracking of the NovaSeq sequencing run and parsing of run statistics into Clarity LIMS, via the Sequencer API.

The validation steps assume that the Illumina NovaSeq 6000 Integration Package v3.5.0 is installed and you have imported the default Clarity LIMS configuration.

Activate Workflow, Create Project, Add and Assign Samples

The following steps set up Clarity LIMS in preparation for running samples through the Library Prep Validation and NovaSeq 6000 v3.5 workflows.

1. On the Configuration tab, under Workflows, activate both the Library Prep Validation and NovaSeq 6000 v3.5 workflows.
   - Library Prep Validation v2.2.0 is available with IPP v2.4.
2. On the Projects and Samples screen, create a project and add samples to it.

3. Assign the samples to the Library Prep Validation workflow. The workflow must be activated before you can assign samples to it.

Library Prep Protocol: Library Prep Validation v2.2.0

This single-step protocol models the library prep required to produce normalized libraries that are ready for the NovaSeq 6000 v3.5 workflow.

Run Library Prep Validation v2.2.0

1. In Lab View, locate the Library Prep Validation v2.2.0 protocol.
   Samples are queued for the Library Prep Validation v2.2.0 step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Placement screen, complete the following actions:
   a. In the Placed Samples area on the right, in the Container field, scan or enter the barcode of the destination container.
   b. Select the samples from the Samples to be Placed area on the left side, and drag them to the container wells on the right side.
   c. Select Add Labels.
5. On the Add Labels screen, the Labels to Add list is populated with labels from the TruSeq HT Adapters v2 (D7-D5) label group. The group is the only label group configured in the integration and is used by default in this step.
   a. Select labels on the left side, and drag them to the container on the right side to assign them to placed samples.
   b. Select Record Details.
6. On the Record Details screen, complete the following actions:
   a. In the Sample Details table, enter values for Normalized Molarity (nM). Type values individually or copy and paste to enter multiple values from a spreadsheet.
   b. The Sequencing Instrument column indicates the instrument to be used for the sequencing run. Select NovaSeq 3.0.
   c. Populate the fields in this column using one of the following methods:
      - To populate fields individually for each sample, select from the drop-down list in each row.
      - To populate all fields at one time, copy and paste from a spreadsheet.

   Alternatively, select inside the table and press Ctrl + A to select all rows. At the top of the table, select Sequencing Instrument and then select NovaSeq 3.0 from the adjacent drop-down list. Select Apply.

   If working with many samples, copy and paste to insert multiple values from a spreadsheet as follows.

   a. Select Copy Samples to copy the sample information from Clarity LIMS to the Clipboard.

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b. Open a blank Excel document and paste the contents of the clipboard into the spreadsheet.

c. In the spreadsheet, enter the Normalized Molarity and Sequencing Instrument values. Select the entire spreadsheet including the headers (Ctrl + A), and copy (Ctrl + C) the information to the Clipboard.

d. Within Clarity LIMS, select the Paste Samples button. Paste the contents of the clipboard (Ctrl + V) into the Update Table window and then select Update to paste the contents into the Sample Details table.

7. Select Next Steps.
   Triggers the Next Steps automation, which sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step.
8. On the Assign Next Steps screen in the Samples table, the Next Step value for all samples is prepopulated with Remove from workflow.

   Do not change this value. If Next Step is not set to Remove from workflow, the routing script does not route samples correctly.

9. Select Finish Step.
   On exit from the step, the Routing Script automation is triggered. This automation assigns samples to the first step of the NovaSeq 6000 v3.5 workflow, Define Run Format (NovaSeq 6000 v3.5). This is the only step in Protocol 1: Run Format (NovaSeq 6000 v3.5).

Protocol 1: Run Format (NovaSeq 6000 v3.5)

This protocol includes a single step, Define Run Format (NovaSeq 6000 v3.5). The step allows for the assignment of per sample values for Loading Workflow Type, Normalized Molarity, Flowcell Type, and Final Loading Concentration (pM). At the end of the step, samples are routed to the NovaSeq Standard or NovaSeq Xp protocol, according to the selected Loading Workflow Type.

Step 1: Run Define Run Format (NovaSeq 6000 v3.5)

1. In Lab View, locate the Run Format (NovaSeq 6000 v3.5) protocol. The samples are queued for the Define Run Format (NovaSeq 6000 v3.5) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Record Details screen in the Sample Details table, populate the following fields (values can vary across samples):
   - Loading Workflow Type—Select NovaSeq Standard or NovaSeq Xp from the drop-down list.
   - Flowcell Type—Select SP, S1, S2, or S4.
   - Final Loading Concentration (pM)—Select from the two preset options: 225 (for PCR-free workflows), 400 (for Nano workflows), or enter a different value.

- Normalized Molarity (nM)—These values are copied over from the previous step. If this column is not populated during library prep, enter the values here.

5. Select Next Steps, which triggers the Next Steps automation and the following actions:
   - Sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step.
   - Calculates the Minimum Molarity.
   - Checks Normalized Molarity value. For samples with no Normalized Molarity value (an empty value not including 0), generates an error message notifying that the field cannot be empty.
   - Compares each sample Normalized Molarity value with the Minimum Molarity value.
6. On the Assign Next Steps screen:
   - In the Sample Details table, the Next Step for all samples is prepopulated with Remove from workflow, regardless of the Loading Workflow Type.

     Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not routed samples correctly.

   - For samples whose Normalized Molarity value is lower than the Minimum Molarity value, the Loading Workflow Type is set to Remove from workflow. Also, a message is recorded in the Warning field for the sample.
     At this point, there are two options:
     - Return to the Record Details screen and adjust the Normalized Molarity value so that it equals or exceeds the Minimum Molarity value. The Loading Workflow Type must be set to NovaSeq Standard or NovaSeq Xp, as applicable.
     - Complete the protocol without correcting the Normalized Molarity value. In this case, the samples in question are removed from the Clarity LIMS workflow.
7. Select Finish Step. The Routing Script automation is triggered:
   - Samples whose Loading Workflow Type is set to Remove from workflow are removed from the Clarity LIMS workflow. (Occurs when the Normalized Molarity value is lower than the Minimum Molarity.)
   - Samples whose Loading Workflow Type is set to NovaSeq Standard are routed to the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.5) step. This is the first step in the NovaSeq Standard protocol.
   - Samples whose Loading Workflow Type is set to NovaSeq Xp are routed to the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.5) step. This is the first step in the NovaSeq Xp protocol.

Protocol 2: NovaSeq Standard (NovaSeq 6000 v3.5)

In this protocol, samples are pooled and added to the library tube in preparation for the NovaSeq run.

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Step 1: Run Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.5)

1. In Lab View, locate the NovaSeq XP (NovaSeq 6000 v3.5) protocol. Samples for the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.5) step are queued.
2. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Pooling screen:
   a. Create a pool by dragging samples into the Pool Creator.

      Only create one pool.

   b. Enter a name for the pool or accept the default name (Pool #1).
   c. Select Record Details.
      After exiting from the Pooling screen, the Validate Inputs Flowcell Type and Single Pool automation is triggered. The automation verifies the following items:
      - All samples in the pool have been assigned the same Flowcell Type.
      - Only one pool has been created.
5. On the Record Details screen in the Step Details area, complete the two required fields and one optional field, as needed:
   - Number of Flowcells to Sequence—Used in volume calculations, to make sure that the volumes are sufficient for the number of times the pool is sequenced.
   - Minimum Per Sample Volume (µl)—Used to calculate how much of each sample is included in the pool. The field is prepopulated with the configured default value 5 µl, but can be edited.
   - % PhiX (2.5 nM) Spike-In—Used to calculate the volume of PhiX v3 control included in the pool for the given percentage of spike-in. The field is optional and can be edited.

     Assuming the default Minimum Per Sample Volume (µl) value of 5, for a given batch:
     - If the smallest Per Sample Volume (µl) value is < 5, Clarity LIMS automatically assigns a value of 5 to the Adjusted Per Sample Volume (µl) field.
     - Clarity LIMS changes the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.
6. In the Sample Details table, select the pool icon to view details on the pool composition.
7. Select Calculate Volumes to trigger the Calculate Volumes automation. This automation performs calculations based on the selected Flowcell Type.
   - Calculates Bulk Pool Volume (µl) value.
   - Sets the value of the Bulk Pool Volume (µl) field.

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- Calculates the PhiX Volume (µl) value if % PhiX (2.5 nM) Spike-In has value.
- Calculates the Per Sample Volume (µl) value to be added to the pool.
- Calculates the Total Sample Volume (µl) value.
- If the Total Sample Volume is less than the Bulk Pool Volume, selects the RSB Volume (µl) value.
- Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
- Populates the Volume of Pool to Denature (µl), NaOH Volume (µl) and Tris-HCl Volume (µl) columns of the Sample Details table. The script sets the values, and is not editable.
- The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains volume information about the pool and the individual samples it contains. Select on the file to download it and then open it in Excel.

8. Select Next Steps to trigger the Set Next Step automation. This automation sets the next step for samples to ADVANCE and advances them to the next step in the protocol, Dilute, and Denature (NovaSeq 6000 v3.5).
   On the Assign Next Steps screen, the next step for samples is set to the next step in the workflow: Dilute and Denature (NovaSeq 6000 v3.5).
9. Select Finish Step.
   At the end of this step, the pool of samples automatically advances to the Dilute and Denature (NovaSeq 6000 v3.5) step.

Step 2: Run Dilute and Denature (NovaSeq 6000 v3.5)

1. In Lab View, locate the NovaSeq Standard (NovaSeq 6000 v3.5) protocol. The pool of samples queued for the Dilute and Denature (NovaSeq 6000 v3.5) step are listed.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work. The Validate Single Input automation is triggered. This automation verifies that only one container is input for the step.
4. On the Placement screen:
   a. Drag the pool into the library tube in the Placed Samples area.
   b. Scan or type the barcode of the library tube into the Library Tube field.
   c. Select Record Details.
   After exiting the Placement screen, the Validate Library Tube Barcode automation makes sure that the library tube barcode conforms to the barcode mask [A-Z]{2}[0-9]{7}-[A-Z]{3}. If it does not, an error message displays. The automation also copies the Flowcell Type and Loading Workflow Type values from step inputs to outputs.
5. On the Record Details screen, the Reagent Lot Tracking section lets you tracks the NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. Add and activate lots for these reagents.

To add and activate reagent lots:

a. In a new browser tab, open an additional instance of Clarity LIMS.
b. Navigate to the Consumables configuration screen.
c. Select the NaOH reagent kit and select New Lot.
d. Enter the lot details.
e. Set the Status of Reagent Lot toggle switch to Active.
f. Select Save.
g. Repeat this process to add and activate Resuspension Buffer and Tris-HCl reagent lots.
h. Return to your original working browser tab and refresh the page.

6. In the Reagent Lot Tracking section, select from the active lots displayed in each drop-down list.
7. The fields displayed on the Record Details screen are used to set up the run and generate the sample sheet. Some of these fields are autopopulated and some must be completed manually.

Table 154 Fields Displayed on Record Details Screen of Denature and Dilute (NovaSeq 6000 v3.5)

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.
BaseSpace Sequence Hub Configuration	Presets • Not Used • Run Monitoring Only • Run Monitoring and Storage
Workflow	Preset • GenerateFASTQ
Workflow Type	Presets • No Index • Single Index • Dual Index • Custom
Index Read 1	Presets • 0 • 6 • 8 • type a value between 0 and 20

Field	Value
Index Read 2	Presets • 0 • 6 • 8 • type a value between 0 and 20
Paired End	Presets • True • False
Read 1 Cycles	Presets • 251* • 151 • 101 • 51 • type a value between 1 and 251 *The value of 251 is only supported for SP flow cell type. For all other flow cell types, the maximum value is 151.
Read 2 Cycles	Presets • 251* • 151 • 101 • 51 • type a value between 0 and 251 *The value of 251 is only supported for SP flow cell type. For all other flow cell types, the maximum value is 151.
Use Custom Read 1 Primer	Select if applicable.
Use Custom Read 2 Primer	Select if applicable.
Use Custom Index Read 1 Primer	Select if applicable.
Reverse Complement Workflow	Select Yes (by default) for v1.5 reagents in NovaSeq Control Software v1.7 and onwards. Select No for v1 reagents.
Use Custom Recipe	Select if applicable.
Custom Recipe Path	If the Use Custom Recipe option is selected, enter the path to the custom recipe file to be used.
UMI - Read 1 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 1. Leave blank if not applicable.

Field	Value
UMI - Read 2 Length	Enter the length of the UMI in Read 2. Leave blank if not applicable.
UMI - Read 1 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.
UMI - Read 2 Start From Cycle	Enter the cycle number that Read 2 starts from. Leave blank if not applicable.
Output Folder	Enter network path for sequencing run folder (eg, `\\networkshare\run_data`)
Samplesheet Format	Presets • V1 • V2
Analysis Software Version	Enter the software version for BCL Convert Analysis for v2 sample sheet. Leave blank if not applicable.
Override Cycles	Enter the Override Cycles value for v2 sample sheet. Leave blank if not applicable.

8. On the Record Details screen, select Validate Run Setup and Generate Sample Sheet. This triggers the automation script, which performs the following actions:
   - Validates the parameters entered on the Record Details screen.
   - Generates the sample sheet and attaches it to the placeholder in the Files area of the Record Details screen.

     The v2 sample sheet generated does not include Cloud and Analysis sections. Contact Illumina Support for information on generating v2 sample sheets with those sections.

   - Select Next Steps.
9. On the Assign Next Steps screen, the Next Step for samples is prepopulated with Remove from workflow. The Routing Script automation expects this value, and requires the value to advance samples to the next step.

   Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.

10. Select Finish Step.
    After exiting the step, the following actions occur:
    - The Routing Script automation is triggered and samples are routed to AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5).
    - In Lab View, the pool of samples is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5) step.

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At this point in the workflow, the user interaction ends. Proceed to AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5).

Do not add samples to the Ice Bucket or start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5) step. The integration adds samples automatically.

Protocol 3: NovaSeq Xp (NovaSeq 6000 v3.5)

In this protocol, samples are pooled and added to lanes on the NovaSeq flow cell. The flow cell type is determined by the option selected in the Define Run Format (NovaSeq 6000 v3.5) step.

Step 1: Run Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.5)

1. In Lab View, locate the NovaSeq Xp (NovaSeq 6000 v3.5) protocol. The samples are queued for the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.5) step.
2. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Pooling screen, create and name a pool as follows.
   a. Create a pool by dragging samples into the Pool Creator.

      Create only one pool.

   b. Enter a name for the pool or accept the default name (Pool #1).
   c. Select Record Details.
5. On exiting the Pooling screen, the Validate Inputs Flowcell Type and Single Pool automation is triggered. The automation verifies the following items:
   - All samples in the pool are assigned the same Flowcell Type.
   - Only one pool was created.
6. On the Record Details screen in the Step Details area, complete the two required fields and one optional field, as needed:
   - Number of Lanes to Sequence—Used in volume calculations to make sure that volumes are sufficient for the number of times the pool is sequenced.
   - Minimum Per Sample Volume (µl)—Used to calculate how much of each sample is included in the pool. The field is prepopulated with the configured default value 5 µl, but can be edited.
   - % PhiX (0.25nM) Spike-In—Used to calculate the volume of PhiX v3 control to be included in the pool for the given percentage of spike-in. The field is optional and can be edited.

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Assuming the default Minimum Per Sample Volume (µl) value of 5, for a given batch:

- If the smallest Per Sample Volume (µl) value is < 5, Clarity LIMS automatically assigns a value of 5 to the samples Adjusted Per Sample Volume (µl) field.
- Clarity LIMS then adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

7. In the Sample Details table, select the pool icon to view details on the pool composition.
8. Select Calculate Volumes. This triggers the Calculate Volumes automation. This automation performs volume calculations based on the selected Flowcell Type, as follows.
   - Calculates Bulk Pool Volume (µl) value.
   - Calculates the Per Sample Volume (µl) value to be added to the pool.
   - Calculates PhiX Volume (ul) value if % PhiX (0.25nM) Spike-In has value.
   - Calculates the Total Sample Volume (µl) value.
   - If the Total Sample Volume is less than the Bulk Pool Volume, calculate the RSB Volume (µl) value.
   - Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
   - Generates the Calculation File (CSV) and attaches it to the step. This file contains volume information about the samples and RSB buffer to add to the pool. Select the file to download it and then open it in Excel.
9. Select Next Steps to trigger the Set Next Step automation. This automation performs the following actions:
   - Copies the Flowcell Type values from the step inputs to the step outputs.
   - Sets the next step for samples to ADVANCE, advancing them to the Dilute, Denature, and ExAmp (NovaSeq 6000 v3.5) step in the protocol.

   On the Assign Next Steps screen, the next step for samples is set to the Dilute, Denature, and ExAmp (NovaSeq 6000 v3.5) step in the workflow.
10. Select Finish Step.
    At the end of this step, the pool of samples automatically advances to the Dilute, Denature, and ExAmp (NovaSeq 6000 v3.5) step.

Step 2: Run Dilute, Denature and ExAmp (NovaSeq 6000 v3.5)

1. In Lab View, locate the NovaSeq Xp (NovaSeq 6000 v3.5) protocol. The pool of samples queued for the Dilute, Denature, and ExAmp (NovaSeq 6000 v3.5) step displays.
2. Add the pool to the Ice Bucket and select View Ice Bucket.

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3. [Optional] On the Ice Bucket screen, set the number of derivatives to create (placed into the flow cell lanes) and select Begin Work.
4. On entry to the Record Details screen, the Calculate Volumes automation is triggered.
   This automation sets the following values based on the selected Flowcell Type:
   - BP Aliquot Volume (µl)
   - NaOH Volume (µl)
   - Tris-HCl Volume (µl)
   - DPX1 Volume (µl)
   - DPX2 Volume (µl)
   - DPX3 Volume (µl)
   - Mastermix per Lane (µl)
   - The automation also populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
   - The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains information about the DPX Mastermix volume and the volume of Mastermix, NaOH, and Tris-HCl to add per working pool (see the next step).
5. On the Record Details screen, the Reagent Lot Tracking section tracks the DPX1, DPX2, DPX3, NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. These reagents must be added.
   To add and activate reagent lots:
   a. In a new browser tab, open an additional instance of Clarity LIMS.
   b. Select one of the reagent kits previously listed and select New Lot.
   c. Enter the lot details.
   d. Set the Status of Reagent Lot toggle switch to Active.
   e. Select Save.
   f. Repeat this process to add and activate the remaining reagent lots.
   g. Return to your original working browser tab and refresh the page.
6. On the Record Details screen:
   - In the Reagent Lot Tracking section, select from the active lots displayed in each drop-down list.
   - In the Step Details area, the DPX1, DPX2, and DPX3 reagent volume values are prepopulated. The script sets the values and they are not editable.
   - In the Sample Details table, select the pool icon to view details on the working pool composition.
     - The BP Aliquot, Mastermix per lane, NaOH, and Tris-HCl volume values for each working pool are populated. The script sets the values and they are not editable.

- The Flowcell Type and Loading Workflow Type columns are populated.
- The working pool number is appended to the bulk pool name, to identify which working pools are derived from the same bulk pool.

7. In the Files area, select the Calculation File (CSV) to open it and view details on the DPX Mastermix volume and the volume of Mastermix, NaOH, and Tris-HCl to add per working pool.
8. Select Next Steps. On the Assign Next Steps screen, the next step is already set to Load to Flowcell (NovaSeq 6000 v3.5).
9. Select Finish Step.

Step 3: Run Load to Flowcell (NovaSeq 6000 v3.5)

1. On the Ice Bucket screen:
   - In the Container Options panel, select the appropriate flow cell type from the Destination Container drop-down list.
   - Select Begin Work.
2. The Validate Inputs and Selected Container automation checks the following:
   - The Flowcell Type field is set to a valid value (SP, S1, S2, or S4) and that each input has the same value for the field.
   - The container type selected matches the value in the Flowcell Type field.
   - The number of outputs matches the number of lanes on the selected flow cell type. If validation fails, an error message reports the number of working pools does not match the number of lanes available on the flow cell.
3. On the Placement screen:
   - Drag one or more pools from the left of the screen over into the Placed Samples area on the right.
   - Scan or type the barcode of the flow cell into the Flowcell field.
   - Select Record Details.

   After exiting the Placement screen, the Validate Flowcell Barcode automation makes sure that the container barcode conforms to the barcode mask for the selected Flowcell Type (as listed in the following table). This automation also copies the Flowcell Type and Loading Workflow Type field values from step inputs to outputs.

Flowcell Type	Barcode Mask
SP	[A-Z0-9]{5}DRX[XY2357]
S1	[A-Z0-9]{5}DRX[XY2357]
S2	[A-Z0-9]{5}DMX[XY2357]
S4	[A-Z0-9]{5}DSX[XY2357]

4. The fields displayed on the Record Details screen are used to set up the run and generate the sample sheet.
   Some of these fields are autopopulated and some must be entered manually. For details, refer to the following table:

   Table 155 Fields Displayed on Record Details Screen of Load to Flowcell (NovaSeq 6000 v3.5) Step

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.
BaseSpace Sequence Hub Configuration	Presets • Not Used • Run Monitoring Only • Run Monitoring and Storage
Workflow	Preset • GenerateFASTQ
Workflow Type	Presets • No Index • Single Index • Dual Index • Custom
Index Read 1	Presets • 0 • 6 • 8 • type a value between 0 and 20
Index Read 2	Presets • 0 • 6 • 8 • type a value between 0 and 20
Paired End	Presets • True • False

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Field	Value
Read 1 Cycles	Presets • 251* • 151 • 101 • 51 • type a value between 1 and 251 *The value of 251 is only supported for SP flow cell type. For all other flow cell types, the maximum value is 151.
Read 2 Cycles	Presets • 251* • 151 • 101 • 51 • type a value between 0 and 251 *The value of 251 is only supported for SP flow cell type. For all other flow cell types, the maximum value is 151.
Use Custom Read 1 Primer	Select if applicable.
Use Custom Read 2 Primer	Select if applicable.
Use Custom Index Read 1 Primer	Select if applicable.
Reverse Complement Workflow	Select Yes (by default) for v1.5 reagents in NovaSeq Control Software v1.7 and onwards. Select No for v1 reagents.
Use Custom Recipe	Select if applicable.
Custom Recipe Path	If you selected the Use Custom Recipe option, enter the path to the custom recipe file to be used.
UMI - Read 1 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 1. Leave blank if not applicable.
UMI - Read 2 Length	Enter the length of the UMI in Read 2. Leave blank if not applicable.
UMI - Read 1 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.
UMI - Read 2 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.

Field	Value
Output Folder	Enter network path for sequencing run folder (eg, `\\networkshare\run_data`)
Library Tube Barcode	Scan the library tube barcode.
Samplesheet Format	Presets • V1 • V2
Analysis Software Version	Enter the software version for BCL Convert Analysis for v2 sample sheet. Leave blank if not applicable.
Override Cycles	Enter the Override Cycles value for v2 sample sheet. Leave blank if not applicable.

5. Select Validate Run Setup and Generate Sample Sheet, then select Next Steps to trigger the automation script, which does the following:
   - Validates the parameters entered on the Record Details screen.
   - Generates the sample sheet and attaches it to the placeholder in the Files area on the Record Details screen.

     The v2 sample sheet generated does not include Cloud and Analysis sections. Contact Illumina Support for information on generating v2 sample sheets with these sections.
6. Select Next Steps.
   On the Assign Next Steps screen, the Next Step field for samples is prepopulated with Mark protocol as complete.
7. Select Finish Step.
   At this point in the workflow, the user interaction ends. The flow cell is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5) step.

Proceed to AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5).

Do not add samples to the Ice Bucket or start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5) step. The integration adds samples automatically.

Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5)

This protocol contains a single fully automated step - AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5).

The integration starts the step automatically and data from the run is parsed back into Clarity LIMS. User interaction is not required, but the various stages can be reviewed in Clarity LIMS (see Review Run Data).

Automations

The NovaSeq Run (NovaSeq 6000 v3.5) step contains two automations:

- Set Next Steps—This automation is disabled (Set to Not Used). By default, the Sequencer API is used to set the next step for output samples.
  The Illumina Preset Protocols (IPP) include a Set Next Steps automation configured on the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5) step. You can use this automation to override the default next step behavior. For details, refer to *NovaSeq 6000 Integration v3.5.0 Configuration and User Interaction* on page 454for details.
- Update Lane Number—This automation is triggered automatically upon entry to the Record Details screen.
  Do not disable or modify this automation to make sure that the Lane Number displays properly.

Review Run Data

Read summary metrics are recorded for the library pool. After the run is complete, open the step and review these metrics on the Next Steps screen in the Step Data section and the Sample Details table.

Step Data Section

The following values populate in the master step fields:

- Run ID
- Run Status
- Output Folder
- Current Cycle
- Current Read
- Loading Workflow Type
- Flow Cell ID
- Flow Cell Side
- Flow Cell Mode
- Flow Cell Part Number*
- Flow Cell Lot Number
- Flow Cell Expiration Date
- Instrument ID
- Instrument Type
- Instrument Control Software Version
- Firmware Version

- RTA Version
- Sequencing Log

Sample Details Table

Summary metrics populate the global fields listed. Values are aggregated across all lanes. Some values (eg, Yield PF (Gb) R1) are summed, while others are averaged.

- Yield PF (Gb) R1
- Yield PF (Gb) R2
- Reads PF (M) R1
- Reads PF (M) R2
- Cluster Density (K/mm^2) R1
- Cluster Density (K/mm^2) R2
- %PF R1
- %PF R2
- % Bases >=Q30 R1
- % Bases >=Q30 R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- % Aligned R1
- % Aligned R2
- % Error Rate R1
- % Error Rate R2

How the Integration Works

The following steps summarize how the Sequencer API integration works. For details on sample sheet generation and the sequence of events involved in the NovaSeq run, refer to *NovaSeq 6000 Integration v3.5.0 Configuration and User Interaction* on page 454.

1. When setting up a run on the NovaSeq 6000 instrument, NVCS sends a request for the run recipe.
    a. The Sequencer API validates that samples and containers are correctly queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5) step.

b. Clarity LIMS sends a JSON response to NVCS. The response includes the run recipe information and a link to download the sample sheet (used with analysis software such as bcl2fastq2).

2. When the run starts, NVCS sends a RunStarted run status request. The Sequencer API then does the following:
   a. Validates reagent kit information in the RunStarted request:
      - Verifies that the reagent kit exists. If the reagent kit does not exist, the Sequencer API creates and enables it on the master step.
      - Verifies that the reagent kit is activated. If the reagent kit is not active, the Sequencer API activates it.
   b. Initiates the step for the queued samples. The step produces one output file placeholder per lane of the flow cell in use, based on the flow cell type.
   c. Records all relevant information from the RunStarted request on the step (such as reagent lots and step fields).
3. At the end of the run, NVCS sends one of the following run status requests:
   - RunCompletedSuccessfully
   - RunEndedByUSer
   - RunErroredOut

   The Sequencer API updates the step with any new information, which includes only the status and cycle/read information.
4. After primary analysis completes, NVCS sends a request containing the parsed run metrics. NVCS sends a request only when the run completed successfully, but the API does not make this assumption and accepts the request regardless of status. The Sequencer API then does the following actions:
   a. Records the metrics into the fields on file placeholder outputs in the LIMS.
   b. Completes the step in Clarity LIMS if the status is RunCompletedSuccessfully. For all other status options, the step remains in progress. This action is the default autocomplete step behavior. For details, refer to *NovaSeq 6000 Integration v3.5.0 Configuration and User Interaction* on page 454.

   > *NovaSeq 6000 Integration v3.5.0 Configuration and User Interaction* on page 454 provides detailed information on how the following components work with the Sequencer API integration:
   >
   > - Sample sheet generation and contents
   > - NovaSeq 6000 run
   > - User authentication and login
   > - Run recipe contents

Troubleshooting

If an automation trigger does not appear to run its corresponding scripts, refer to the following sections in the Clarity LIMS API documentation:

- *Troubleshooting Automated Informatics*
- *Troubleshooting Automation in the API*

If an error occurs that does not provide direction on how to proceed, complete the following steps:

1. Confirm the version of the installed Illumina NovaSeq 6000 Integration Package by running the following command on the Clarity LIMS server command line:

```
rpm -qa | grep -i novaseq
```

2. If the error is related to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5) step, review the log file information. For details, refer to *Logging* on page 533.

If the automated run step starts but does not complete, proceed as follows.

1. Use one of the following methods to open the in progress step in Clarity LIMS:
   - Log in to the default user account.
   - In Lab View, find the step in the Recent Activities pane.
   - Search for the step in Clarity LIMS, using either the Library Tube, or Flowcell barcode as the search term. The steps depend on whether the search is a NovaSeq Standard or NovaSeq Xp run.
2. On the Record Details screen, the Sequencing Log multiline text field contains logging information. If you are unable to reach the Record Details screen, or if the Sequencing Log field does not contain enough information to resolve the issue, review the `sequencer-api.log` file. For details, refer to *Logging* on page 533.
3. Contact Illumina Support. Provide the relevant information from the troubleshooting steps already performed.

Logging

In addition to updating the Sequencing Log multiline text field on the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.5) step, the Sequencer API writes a detailed log file to the following location:

`/opt/gls/clarity/tomcat/current/logs/sequencer-api.log`

Log messages include the Library Tube ID and Flowcell ID whenever the messages are related to a sequencing run-related request. In the LIMS workflows, these IDs are recorded as container names.

Some log messages are not directly related to a sequencing run request, such as downloading the sample sheet. In this case, the file LIMS ID and file name are included in log messages. The sample sheet downloaded by the sequencing system has a name that includes the sequencing container ID (Library Tube for Standard or Flowcell for Xp). The relevant container name search finds most the log

messages for this request. However, if all messages for downloading a file are found, the LIMS ID of the file must be known. The LIMS ID is found in the Clarity LIMS API and, for sample sheets used in a run, in the recipe response (also included in the log file).

Example lines from the log file:

```
2021-06-19 21:32:19.705 INFO --- [] SequencerAPIApplication : Started
SequencerAPIApplication in 7.026 seconds (JVM running for 15.964)
...
2021-06-19 21:34:44.564 INFO --- [Library Tube Id=NV1234567-LIB, Flow Cell
Id=RM123DSXX] LimsApiLookupUtil : Searching for containers with name 'NV1234567-
LIB' or 'RM123DSXX'
....
2021-06-19 21:35:27.158 INFO --- [] FileService : Successfully retrieved file
'NV1234567-LIB.csv' with LIMS ID 40-51 through the API.
```

NovaSeq 6000 Integration v3.5.0 Manual Upgrade

NovaSeq Integration Package v3.5.0 contains modifications to the existing automations from NovaSeq Integration Package v3.4.0.

If you do not want to upgrade the existing NovaSeq Sequencing workflow to the latest version through Illumina Preset Protocols (IPP), you can update the workflow configuration manually by completing the following steps.

Update Validate Flowcell Barcode Automation on Load to Flowcell (NovaSeq 6000 v3.3) Step

Update the Validate Flowcell Barcode automation on the Load to Flowcell (NovaSeq 6000 v3.3) step as follows.

1. From Configuration, select the Automation tab.
2. Under the Step Automation tab, select the Validate Flowcell Barcode automation enabled on the Load to Flowcell (NovaSeq 6000 v3.3) step.
3. Update the existing command line with the following script:
   Changes to the existing command line are in red.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-exp 'if (input.::Flowcell Type:: == ::SP:: && !output.container.name.matches(
::[A-Z0-9]{5}DRX[XY2357]:: ) ) {fail ( ::Invalid Flowcell Barcode. Please verify
and try again.:: ) } ; if (input.::Flowcell Type:: == ::S1:: &&
!output.container.name.matches( ::[A-Z0-9]{5}DRX[XY2357]:: ) ) {fail ( ::Invalid
Flowcell Barcode. Please verify and try again.:: ) } ; if (input.::Flowcell
```

```
Type:: == ::S2:: && !output.container.name.matches( ::[A-Z0-9]{5}DMX[XY2357]:: )
) {fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: ) } ; if
(input.::Flowcell Type:: == ::S4:: && !output.container.name.matches( ::[A-Z0-9]
{5}DSX[XY2357]:: ) ) {fail ( ::Invalid Flowcell Barcode. Please verify and try
again.:: ) } ; output.::Flowcell Type:: = input.::Flowcell Type:: ;
output.::Loading Workflow Type:: = input.::Loading Workflow Type::' \
-log {compoundOutputFileLuid1}"
```

4. Select Save.

Update Validate Library Tube Barcode Automation on Dilute and Denature (NovaSeq 6000 v3.3) Step

Update the Validate Library Tube Barcode automation on the Dilute and Denature (NovaSeq 6000 v3.3) step as follows.

1. From Configuration, select the Automation tab.
2. Under the Step Automation tab, select the Validate Library Tube Barcode automation enabled on the Dilute and Denature (NovaSeq 6000 v3.3) step.
3. Update the existing command line as follows.
   Changes to the existing command line are in red.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
script:evaluateDynamicExpression -exp 'if (!output.container.name.matches( ::[A-
Z]{2}[0-9]{7}-[A-Z]{3}:: ) ) {fail ( ::Invalid Library Tube Barcode. Please
verify and try again.:: ) } ; output.::Flowcell Type:: = input.::Flowcell Type::
; output.::Loading Workflow Type:: = input.::Loading Workflow Type::' -log
{compoundOutputFileLuid1}"
```

4. Select Save.

NovaSeq 6000 Integration v3.5.0 Release Notes

These release notes describe the key changes to software components for the Clarity LIMS NovaSeq 6000 Integration Package.

Compatibility

This version of the integration package is compatible with the following software:

- Clarity LIMS v5.1 and later
- NGS Extensions Package v5.22 and later
- Illumina Preset Protocols (IPP) v2.4.0 and later
- NovaSeq Control Software (NVCS) v1.8.0
- Illumina bcl2fastq v2.20

New Features

Features provided with this release of the integration package are as follows.

- Fixes the following vulnerabilities:
  - Code injection (CVE-2022-42889)
  - Buffer management errors (CVE-2020-36518, CVE-2022-38752, CVE-2022-38751, CVE-2022-38749)
  - Resource exhaustion (CVE-2022-42003, CVE-2022-42004)
  - Uncontrolled resource consumption (CVE-2022-25857)

Defects Fixed

Fixed the Validate Library Tube and Validate Flowcell Barcode automation scripts to accept only barcodes that are uppercased in the NovaSeq 6000 workflow.

Known Issues

None

NovaSeq 6000 Integration v3.4.0 Configuration and User Interaction

The Illumina NovaSeq 6000 Integration Package v3.4.0 supports the integration of Clarity LIMS to Illumina NovaSeq 6000 instruments. This integration supports Clarity v5.1 and later.

For instructions on validating and troubleshooting the Illumina NovaSeq 6000 Integration, see *NovaSeq 6000 Integration v3.4.0 Validation and Troubleshooting* on page 596.

> The configuration provided in this integration has been established to support NovaSeq 6000 lab processes. Any configuration changes to protocols or workflows - including renaming protocols, steps, and fields - could break process.

Overview

The integration includes the following features:

- Preconfigured NovaSeq 6000 v3.3 workflow that maps to lab protocols and instrument runs.
- The following preconfigured protocols:
  - Run Format (NovaSeq 6000 v3.3)
  - NovaSeq Standard (NovaSeq 6000 v3.3) (supports the loading of pooled libraries into a library tube)
  - NovaSeq Xp (NovaSeq 6000 v3.3) (supports individual lane loading on the NovaSeq)
  - AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.3)

- Automated generation of v1 sample sheet for use with bcl2fastq2 v2.20 analysis software or v2 sample sheet. This file is automatically uploaded to the instrument via the API.
- Automatic validation of run setup information. This information is used to create the run recipe and initiate the run.
- Automated tracking of the NovaSeq sequencing run and parsing of run statistics into Clarity LIMS, including the following features:
  - Progress and metrics of sequencing run
  - Per-instrument sequencing runs (tracked as part of the Run ID field value)
  - Sequencing run parameters
  - Sequencing run data directory location
  - Real-Time Analysis v3 (RTA3) version and other run-specific information
  - [Optional] Preconfigured Library Prep Validation v2.0.4 workflow used for validation purposes only. The workflow contains a single-step protocol that models the library prep required to produce normalized libraries that are ready for the NovaSeq 6000 v3.3 workflow. See the *NovaSeq 6000 Integration v3.4.0 Validation and Troubleshooting* on page 596. The Library Prep Validation v2.0.4 workflow is available in IPP v2.3 Package.

Prerequisites and Assumptions

It is assumed that samples enter the NovaSeq 6000 v3.3 workflow as normalized libraries. It is assumed that the following steps have completed before samples are assigned to the workflow:

- Samples have been accessioned into the Clarity LIMS.
- Samples have been run through QC and library prep.
- Samples have been normalized, and the value is captured in a field called Normalized Molarity (nM).

For more information on sample accessioning, refer to *Sample Accessioning* and *Upload and Modify Samples* in the Getting Started section of the Clarity LIMS Core Functionality documentation.

You can assign samples to workflows automatically, using a routing script, or manually—from the Projects & Samples dashboard. Refer to *Assign and Process Samples* in the Clarity LIMS Core Functionality documentation.

Workflows, Protocols, and Steps

The Illumina NovaSeq 6000 Integration Package v3.4.0 includes the following workflows:

- NovaSeq 6000 v3.3
- Library Prep Validation v2.0.4 (optional, but recommended for validation purposes)

The following table describes the protocols and steps included in these workflows.

Table 156 NovaSeq 6000 Integration Package v3.4 Protocols and Steps

Protocol Name	Protocol Purpose	Steps Included in Protocol
	Library Prep Validation v2.0.4 Workflow	
Library Prep Validation v2.0.4	Included for validation purposes only, this protocol models the library prep steps required to advance samples to the Run Format (NovaSeq 6000 v3.3) protocol. The protocol contains a single step - Library Prep Validation v2.0.4. After this step, a routing script sends the samples to the first step of the NovaSeq 6000 v3.3 workflow - Define Run Format (NovaSeq 6000 v3.3).	1. Library Prep Validation v2.0.4
	NovaSeq 6000 v3.3 Workflow	
Run Format (NovaSeq 6000 v3.3)	Allows for the assignment of per sample values for: • Loading Workflow Type: Select either NovaSeq Standard or NovaSeq Xp. • Normalized Molarity: Enter a value for each sample. • Flowcell Type: Select from options SP, S1, S2, or S4. • Final Loading Concentration (pM): Select from options 225 (PCR-free workflows) or 400 (Nano workflows), or enter a different value. Compares the Normalized Molarity value of each sample with the Minimum Molarity value. Routing script sends samples to the NovaSeq Standard or NovaSeq Xp protocol, according to the selected Loading Workflow Type. Samples with Normalized Molarity less than Minimum Molarity are removed from the workflow.	1. Define Run Format (NovaSeq 6000 v3.3)

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Protocol Name	Protocol Purpose	Steps Included in Protocol
NovaSeq Standard (NovaSeq 6000 v3.3)	Samples are pooled and added to the library tube in preparation for the NovaSeq run. The run setup information is validated and a sample sheet is generated. Routing script sends the library tube to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.3) protocol.	1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.3) 2. Dilute and Denature (NovaSeq 6000 v3.3)
NovaSeq Xp (NovaSeq 6000 v3.3)	Samples are pooled and added to lanes on the NovaSeq flow cell. The flow cell type is determined by the option selected in the Define Run Format (NovaSeq 6000 v3.3) step. The run setup information is validated and a sample sheet is generated. Routing script sends flow cell to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.3) protocol.	1. Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.3) 2. Dilute, Denature, and ExAmp (NovaSeq 6000 v3.3) 3. Load to Flowcell (NovaSeq 6000 v3.3)
AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.3)	All samples complete the workflow by going through this protocol. This protocol contains one fully automated step. Do not add samples to the Ice Bucket or start the step. The integration starts the step automatically.	1. AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.3)

Validation Workflow

The Library Prep Validation v2.0.4 workflow allows for validation of the system after installation is complete. For details, see *NovaSeq 6000 Integration v3.4.0 Validation and Troubleshooting* on page 596.

Step Automations

The following table lists the step automations included in the NovaSeq 6000 v3.3 workflow. Automation details are provided in the documentation for each step.

For details on the validation workflow, see *NovaSeq 6000 Integration v3.4.0 Validation and Troubleshooting* on page 596.

Table 157 NovaSeq 6000 v3.3 workflow step automations

Protocol	Step	Automation
1. Run Format (NovaSeq 6000 v3.3)	1. Define Run Format (NovaSeq 6000 v3.3)	Routing Script Set Next Steps
2. NovaSeq Standard (NovaSeq 6000 v3.3)	1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.3)	Calculate Volumes Set Next Step Validate Inputs Flowcell Type and Single Pool Validate Unique Indexes (Not used. This functionality is handled by the core Clarity LIMS configuration for pooling.)
	2. Dilute and Denature (NovaSeq 6000 v3.3)	Routing Script Check Library Tube Barcode Validate Run Setup and Generate Sample Sheet Validate Single Input
3. NovaSeq Xp (NovaSeq 6000 v3.3)	1. Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.3)	Calculate Volumes Set Next Step Validate Inputs Flowcell Type and Single Pool Validate Unique Indexes (Not used. This functionality is handled by the core Clarity LIMS configuration for pooling.)
	2. Dilute, Denature, and ExAmp (NovaSeq 6000 v3.3)	Calculate Volumes Set Next Step Validate Inputs Flowcell Type
	3. Load to Flowcell (NovaSeq 6000 v3.3)	Validate Flowcell Barcode Validate Inputs and Selected Container Validate Run Setup and Generate Sample Sheet

Protocol	Step	Automation
4. AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.3)	1. AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.3)	Update Lane Number Set Next Steps (Not used. By default, this functionality is determined by the Sequencer API. If necessary, enable the automation and use it to override the next step behavior used by the Sequencer API.)

Terminology

This integration package supports BaseSpace Clarity LIMS v5.1 and later.

As of Clarity LIMS v5, the term user-defined field (UDF) has been replaced with custom field in the user interface. However, the API resource is still called UDF.

There are two types of custom fields:

- Master step fields—Configured on master steps. Master step fields only apply to the following:
  - The master step on which the fields are configured.
  - The steps derived from those master steps.
- Global fields—Configured on entities (eg, submitted sample, derived sample, measurement, etc.). Global fields apply to the entire Clarity LIMS system.

Protocol 1: Run Format (NovaSeq 6000 v3.3)

This protocol sets the Loading Workflow Type and allows the choice of the appropriate Flowcell Type and Final Loading Concentration (pM). After the protocol, a routing script sends the normalized libraries to either the NovaSeq Standard (NovaSeq 6000 v3.3) or the NovaSeq Xp (NovaSeq 6000 v3.3) protocol.

This protocol contains one step: Define Run Format (NovaSeq 6000 v3.3).

Step 1. Define Run Format (NovaSeq 6000 v3.3)

Step input: NTP (normalized libraries)

Step output: None

Set Next Steps Automation

Automatically triggered on exit of the Record Details screen, this automation completes the following actions:

- Sets the next step for samples to REMOVE:

```
nextStep = ::REMOVE::
```

- Calculates the Minimum Molarity using the following formula:

```
input.::Minimum Molarity (nM):: = (5 * input.::Final Loading Concentration
(pM)::)/1000
```

- Checks Normalized Molarity value. For samples with no Normalized Molarity value (ie, empty value, not including 0), it generates an error message informing that the field cannot be empty:

```
if (!input.hasValue(::Normalized Molarity (nM)::)) {
  fail(::The Normalized Molarity cannot be empty.::) ;
}
```

- Compares each sample Normalized Molarity value with the Minimum Molarity value. If Normalized Molarity value is lower than the Minimum Molarity value, it sets the samples Loading Workflow Type to [Remove from workflow] and records a message in the Warning field for the sample:

```
else if (input.::Normalized Molarity (nM):: < input.::Minimum Molarity (nM)::) {
  input.::Warning:: = ::The Normalized Molarity is too low.:: ;
  input.::Loading Workflow Type:: = ::[Remove from workflow]:: ; }
else { input.::Warning:: = ::not applicable:: }
```

At this point there are two options:

- Correct the Normalized Molarity value on the Record Details screen. Edit the Loading Workflow Type field and set it to NovaSeq Standard or NovaSeq Xp, as applicable.
- Complete the protocol without correcting the Normalized Molarity value. In this case, those samples are removed from the workflow.

Routing Script Automation

Automatically triggered on exit of the step, this automation invokes the changeWorkflow script, which routes step inputs appropriately.

- Samples with Loading Workflow Type field value = NovaSeq Standard are routed to the NovaSeq 6000 v3.3 workflow and queued for the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.3) step.
- Samples with Loading Workflow Type field value = NovaSeq Xp are routed to the NovaSeq 6000 v3.3 workflow and queued for the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.3) step.

Default automation command line is as follows:

```
bash -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar
-u {username} -p {password} -i {stepURI:v2} -l {compoundOutputFileLuid0}
script:changeWorkflow \
\
```

```
--FIELD_NAME 'Loading Workflow Type' \
--FIELD_VALUE 'NovaSeq Standard' \
--WORKFLOW 'NovaSeq 6000 v3.3' \
--STEP 'Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.3)' \
--INPUTS_OR_OUTPUTS 'INPUTS' \
\
--FIELD_NAME 'Loading Workflow Type' \
--FIELD_VALUE 'NovaSeq Xp' \--WORKFLOW 'NovaSeq 6000 v3.3' \
--STEP 'Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.3)' \
--INPUTS_OR_OUTPUTS 'INPUTS'"
```

Master Step Fields

There are four fields defined on the Define Run Format (NovaSeq 6000 v3.3) master step.

The following table lists field configuration details.

Table 158 Define Run Format (NovaSeq 6000 v3.3) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Comment	Multiline Text	• Required: No • Read Only: No	None
Flowcell Type	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	Presets • SP • S1 • S2 • S4
Instruction	Text	• Required: No • Read Only: Yes	Default • Add Flowcell Type and Loading Workflow Type below
Loading Workflow Type	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	Presets • NovaSeq Standard • NovaSeq Xp

Global Fields

The following table lists the global custom fields that are configured to display on the Define Run Format (NovaSeq 6000 v3.3) step.

Table 159 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
Adjusted Per Sample Volume (µl)	Numeric	Required: No Read Only: Yes	Decimal Places Displayed: 2
Final Loading Concentration (pM)	Numeric Dropdown	Required: Yes Read Only: No Custom Entries: Yes	Decimal Places Displayed: 0 Presets • 225 • 400
Flowcell Type	Text Dropdown	Required: Yes Read Only: No Custom Entries: No	Presets • SP • S1 • S2 • S4
Loading Workflow Type	Text Dropdown	Required: Yes Read Only: No Custom Entries: No	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
Minimum Molarity (nM)	Numeric	Required: No Read Only: No	Decimal Places Displayed: 2
Normalized Molarity (nM)	Numeric	Required: No Read Only: No	Decimal Places Displayed: 2
Per Sample Volume (µl)	Numeric	Required: No Read Only: Yes	Decimal Places Displayed: 2
Warning	Text Dropdown	Required: No Read Only: Yes Custom Entries: Yes	The Normalized Molarity (nM) is too low. Not applicable

User Interaction

Tefer to Run Define Run Format (NovaSeq 6000 v3.3) step section in *NovaSeq 6000 Integration v3.4.0 Validation and Troubleshooting* on page 596.

Protocol 2: NovaSeq Standard (NovaSeq 6000 v3.3)

Samples are routed to this protocol if their Loading Workflow Type value is set to NovaSeq Standard. Samples are pooled and added to a library tube in preparation for the NovaSeq run.

At the end of this protocol, a routing script sends the library tube to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.3) protocol.

This protocol contains two steps:

- Step 1: Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.3)
- Step 2: Dilute and Denature (NovaSeq 6000 v3.3)

Step 1: Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.3)

In this step, libraries are placed manually into a single pool. Resuspension buffer and reagents are added.

Create only one pool per step.

Step input: NTP (normalized libraries)

Step output: Bulk pool

Validate Unique Indexes automation is not used. This functionality is handled by the core Clarity LIMS configuration for pooling.

Validate Inputs Flowcell Type and Single Pool Automation

Automatically triggered on exit of the Pooling screen, this automation completes the following actions:

- Checks that all samples in the pool have the same Flowcell Type assigned to them.
- Checks that only one pool has been created.
- Changes the value of error and logging messages to reference the type of pool passed as bulk pool.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar \
script:validate_flowcell_for_input_pools \
  -i {stepURI:v2} \
  -u {username} \
  -p {password} \
  -l {compoundOutputFileLuid1} \
  -validateSingleOutput true \
  -poolType bulk"
```

Calculate Volumes Automation

Automatically triggered when selecting the Calculate Volumes button on the Record Details screen, this automation completes the following actions:

- Calculates the number of samples in the pool.

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: + 1
```

- Sets the value of the Bulk Pool Volume (µl), and PhiX Volume (µl) field based on the selected Flowcell Type.

```
step.::PhiX Volume (ul):: = !step.hasValue(::% PhiX (0.25nM) Spike-In::) ? 0 :
step.::% PhiX (0.25nM) Spike-In::;
if (input.::Flowcell Type:: == ::SP::) {
step.::Bulk Pool Volume (ul):: = step.'Number of Lanes to Sequence' * 18 + 30;
step.::PhiX Volume (ul):: *= 1.9;
};
if (input.::Flowcell Type:: == ::S1::) {
step.::Bulk Pool Volume (ul):: = step.'Number of Lanes to Sequence' * 18 + 30;
step.::PhiX Volume (ul):: *= 1.9;
};
if (input.::Flowcell Type:: == ::S2::) {
step.::Bulk Pool Volume (ul):: = step.'Number of Lanes to Sequence' * 22 + 30;
step.::PhiX Volume (ul):: *= 1.9;
};
if (input.::Flowcell Type:: == ::S4::) {
step.::Bulk Pool Volume (ul):: = step.'Number of Lanes to Sequence' * 30 + 30;
step.::PhiX Volume (ul):: *= 2.2;
};
if (step.::PhiX Volume (ul):: == 0) {
step.::PhiX Volume (ul):: = ::::;
};
```

- Calculates the Per Sample Volume (µl) to be added to the pool.

```
input.::Per Sample Volume (µl):: = ( ( (input.::Final Loading Concentration
(pM):: * 5 / 1000) / input.::Normalized Molarity (nM):: ) * step.::Bulk Pool
Volume (µl):: ) / step.::Number of Samples in Pool::
```

> To ensure accurate pipetting of each sample in a pool for sequencing, the Per Sample Volume (µl) value must be equal to or higher than the Minimum Per Sample Volume (µl) (default value, set at 5, may be edited).
>
> Assuming the default Minimum Per Sample Volume (µl) value of 5, completes the following steps for a given batch:
>
> 1. If the smallest Per Sample Volume (µl) value is less than 5, automatically assigns a value of 5 to the sample Adjusted Per Sample Volume (µl) field.
> 2. Adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

- Calculates the Total Sample Volume (µl) field value.

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```
step.::Total Sample Volume (µl):: = step.::Total Sample Volume (µl):: +
input.::Adjusted Per Sample Volume (µl)::
```

- If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) field value.

```
if (step.::Total Sample Volume (µl):: >= step.::Bulk Pool Volume (µl)::)
{output.::RSB Volume (µl):: = 0}
else {output.::RSB Volume (µl):: = step.::Bulk Pool Volume (µl):: - step.::Total
Sample Volume (µl)::} ;
```

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs.

```
output.::Flowcell Type:: = input.::Flowcell Type:: ;
output.::Loading Workflow Type:: = input.::Loading Workflow Type:: ;
```

- Uses the NovaSeq_Standard_Bulk_Pool1.csv, NovaSeq_Standard_Bulk_Pool2.csv, and NovaSeq_Standard_Bulk_Pool3.csv template files to generate a single CSV file containing information about the pool and the samples it contains. The generated file is available for download on the Step Setup screen of Dilute and Denature (NovaSeq 6000 v3.3).

```
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} \ script:driver_file_generator \
  -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Standard_Bulk_
Pool1.csv \
  -o 1.csv \ script:driver_file_generator \
  -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Standard_Bulk_
Pool2.csv \
  -o 2.csv \ script:driver_file_generator \
  -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Standard_Bulk_
Pool3.csv \
  -o 3.csv \
&& cat 1.csv 2.csv 3.csv > {compoundOutputFileLuid0}.csv
```

- Resets the Total Sample Volume (ul) and Number of Samples in Pool field values so that the automation is idempotent.

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: - 1;
step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: -
input.::Adjusted Per Sample Volume (ul)::
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, the following automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol, Dilute, and Denature (NovaSeq 6000 v3.3):

```
nextStep = ::ADVANCE::
```

Master Step Fields

There are seven fields defined on the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.3) step.

The following table lists configuration details.

Table 160 Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.3) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
% PhiX (2.5 nM) Spike-In	Numeric	Required: No Read Only: No	Range = 0–100 Decimal Places Displayed: 0
Bulk Pool Volume (µl) (for calculation purposes, not displayed)	Numeric	Required: No Read Only: No	Decimal Places Displayed: 2
Number of Flowcells to Sequence	Numeric	Required: Yes Read Only: No	Range = 1–10 Decimal Places Displayed: 0
Minimum Per Sample Volume (µl)	Numeric	Required: Yes Read Only: No	Decimal Places Displayed: 2 Default • 5
Number of Samples in Pool (for calculation purposes, not displayed)	Numeric	Required: No Read Only: No	Decimal Places Displayed: 0 Default • 0
PhiX Volume (µl) (for calculation purposes, not displayed)	Numeric	Required: No Read Only: No	Decimal Places Displayed: 2

Field Name	Field Type	Options	Additional Options and Dropdown Items
Total Sample Volume (µl) (for calculation purposes, not displayed)	Numeric	Required: No Read Only: No	Decimal Places Displayed: 2 Default • 0

Global Fields

The following table lists the global custom fields that are configured to display on the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.3) step.

Table 161 Global Custom Fields Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
Flowcell Type	Text Dropdown	Required: Yes Read Only: No Custom Entries: No	Presets • SP • S1 • S2 • S4
Loading Workflow Type	Text Dropdown	Required: Yes Read Only: No Custom Entries: No	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
NaOH Volume (µl)	Numeric	Required: No Read Only: Yes	Decimal Places Displayed: 2
RSB Volume (µl)	Numeric	Required: No Read Only: Yes	Decimal Places Displayed: 2
Tris-HCl Volume (µl)	Numeric	Required: No Read Only: Yes	Decimal Places Displayed: 2

Field Name	Field Type	Options	Additional Options and Dropdown Items
Volume of Pool to Denature (µl) Used in Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.3) step only. Displays on Record Details screen and in the generated CSV file.	Numeric	Required: No Read Only: Yes	Decimal Places Displayed: 0

User Interaction

Refer to Run Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.3) step section in *NovaSeq 6000 Integration v3.4.0 Validation and Troubleshooting* on page 596.

Step 2: Dilute and Denature (NovaSeq 6000 v3.3)

In this step, pooled samples are denatured and diluted by the addition of NaOH, Tris-HCl, and Resuspension Buffer (RSB). Manually place the pooled samples into the library tube for the NovaSeq run.

In addition, this step validates the run setup information and generates the sample sheet file.

Step input: Bulk pool

Step output: Library tube

Validate Single Input Automation

Automatically triggered at the beginning of the step, this automation checks that there is only one container input to the step.

```
script:validateSampleCount -min 1 -max 1
```

Validate Library Tube Barcode Automation

Automatically triggered on exit of the Placement screen, this automation completes the following actions:

- Validates the library tube barcode to ensure it conforms to the barcode mask [A-Za-z]{2}[0–9]{7}-[A-Za-z]{3}:

```
if (!output.container.name.matches(::[A-Za-z]{2}[0-9]{7}-[A-Za-z]{3}::)) {
fail(::Invalid Library Tube Barcode. Verify and try again.::)
}
```

- Copies Flowcell Type and Loading Workflow Type field values from step inputs to outputs.

```
output.::Flowcell Type:: = input.::Flowcell Type:: ;
output.::Loading Workflow Type:: = input.::Loading Workflow Type::
```

Validate Run Setup and Generate Sample Sheet Automation

The script definition portion of Validate Run Setup has been moved into the Validation Script custom field due to the 4000 character limit.

- If modification is required on Validate Run Setup script, perform the changes directly on the Validation Script custom field default value.
- The script definition portion of the Generate Sample Sheet remains untouched.

Triggered by a button on the Record Details screen, this automation completes the following actions:

- Copies the Flowcell Type from the step input to the Run Mode field (hidden):

```
step.::Run Mode:: = input.::Flowcell Type::
```

- Validates the parameters entered on the Record Details screen, which is used to set up the run and generate the sample sheet file. Refer to *Respond to Validation / Recipe Request Call from Instrument* on page 584 for more details.
  - Experiment Name may only contain alphanumeric, dash, or underscore characters. Spaces are not permitted.
  - When Workflow Type = No Index, both Index Read 1 and Index Read 2 must be zero.
  - When Workflow Type = Single Index, Index Read 1 must be greater than zero, and Index Read 2 must be zero.
  - When Workflow Type = Dual Index, both Index Read 1 and Index Read 2 must be greater than zero.

```
if(!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) {
fail(::Experiment Name contains prohibited characters. Allowed characters are:
a-z, A-Z, 0-9, -, and _::)
};
if (step.::Workflow Type:: == ::No Index::) {
if (step.::Index Read 1:: != 0 || step.::Index Read 2:: != 0) {
fail(::Index Read 1 and Index Read 2 must be 0 if No Index is selected.::)
}
} else if (step.::Workflow Type:: == ::Single Index::) {
if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) {
fail(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if Single
Index is selected.::)
}
```

```
} else {
if (step.::Index Read 1:: == 0 || step.::Index Read 2:: == 0) {
fail(::Index Read 1 and Index Read 2 must be greater than 0 if Dual Index is
selected.::)
}
};
```

- Checks the Paired End and Read 2 Cycles field values.
  - If Paired End = True and Read 2 Cycles value is zero, generates an error.
  - If Paired End = False, Read 2 Cycles must be zero and UMI - Read 2 Length and UMI - Read 2 Start From Cycle must be empty.

```
if (step.::Paired End::.toBoolean()) {
if (step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) {
fail(::Read 1 Cycles and Read 2 Cycles must be greater than 0 if Paired End is
True.::)
}
} else {
if (step.::Read 2 Cycles:: != 0) {
fail(::Read 2 Cycles must be 0 if Paired End is False.::)
};
if (step.hasValue(::UMI - Read 2 Length::) || step.hasValue(::UMI - Read 2 Start
From Cycle::)) {
fail(::UMI - Read 2 Length and UMI - Read 2 Start From Cycle cannot be defined
if Paired End is False.::)
}
};
```

- Checks the Flowcell field value.
  - If Flowcell Type value is not SP, checks that the values of Read 1 Cycles and Read 2 Cycles are each 151 or less. If the value is greater than 151, an error message is generated.

```
if (input.::Flowc ell Type::!=::SP:: && step.::Read 1 Cycles:: > 151) {
fail(::Read 1 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
};
if (input.::Flowcell Type::!=::SP:: && step.::Read 2 Cycles:: > 151) {
fail(::Read 2 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
};
```

- Validates UMI Settings.
  - If UMI - Read 1 Length has value, then UMI - Read 1 Start From Cycle must have value.
  - If UMI - Read 1 Length has no value, then UMI - Read 1 Start From Cycle must not have value.
  - If UMI - Read 2 Length has value, then UMI - Read 2 Start From Cycle must have value.

- If UMI - Read 2 Length has no value, then UMI - Read 2 Start From Cycle must not have value.
- If UMI - Read 1 Length has no value, then UMI - Read 2 Length must not have value.
- If UMI - Read 1 Start From Cycle has no value, then UMI - Read 2 Start From Cycle must not have value.

```
if (step.hasValue(::UMI - Read 1 Length::) && !step.hasValue(::UMI - Read 1
Start From Cycle::)) {
fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 1
Length is greater than 0.::)
};
if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 1
Start From Cycle::)) {
fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 1 Start From
Cycle is greater than 0.::)
};
if (step.hasValue(::UMI - Read 2 Length::) && !step.hasValue(::UMI - Read 2
Start From Cycle::)) {
fail(::UMI - Read 2 Start From Cycle must be greater than 0 if UMI - Read 2
Length is greater than 0.::)
};
if (!step.hasValue(::UMI - Read 2 Length::) && step.hasValue(::UMI - Read 2
Start From Cycle::)) {
fail(::UMI - Read 2 Length must be greater than 0 if UMI - Read 2 Start From
Cycle is greater than 0.::)
};
if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 2
Length::)) {
fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 2 Length is
greater than 0.::)
};
if (!step.hasValue(::UMI - Read 1 Start From Cycle::) && step.hasValue(::UMI -
Read 2 Start From Cycle::)) {
fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 2
Start From Cycle is greater than 0.::)
};
```

- Validate Analysis Software Version to ensure it is a valid version string.

```
if (step.hasValue(::Analysis Software Version::) && !step.::Analysis Software
Version::.matches(::^(\\d+)(\\.\\d+)*$::)) {
fail(::Analysis Software Version contains prohibited characters. Allowed
characters are 0-9 and period. It shall start and end with numbers and separated
by single period e.g. 3.8.4::)
```

```
};
```

- Validate Override Cycles value to only contain Y, N, I, U, 0-9, and semi-colon characters.

```
if (step.hasValue(::Override Cycles::) && !step.::Override Cycles::.matches(::
[YNIU0-9;]+::)) {
fail(::Override Cycles contains prohibited characters. Allowed characters are:
Y, N, I, U, 0-9 and ;. Example: N1Y150;I8;I7N1;Y141U10.::)
};
```

- Outputs the Settings Header in sample sheet if UMI - Read 1 Length or UMI - Read 2 Length have value.

```
step.::Settings Header:: = step.hasValue(::UMI - Read 1 Length::) ||
step.hasValue(::UMI - Read 2 Length::) ? ::[Settings]:: : ::::;
```

- Sets the next step for samples to REMOVE:

```
nextStep = ::REMOVE::
```

- Generates the sample sheet and attaches it to the step. (Refer to *Sample Sheet Generation* on page 582 for more information.) Sample sheet template used depends on the selected value of Samplesheet Format and/or Reverse Complement Workflow.

```
&& if [ '{udf:SampleSheet Format}' == 'V2' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Samplesheet_V2.csv'; \
elif [ '{udf:Reverse Complement Workflow}' == 'true' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Reverse_Complement_Samplesheet.csv'; \
else echo 'UDF Reverse Complement Workflow is empty or undefined.'; exit -1; fi
\
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/$templateFilename \
-o {compoundOutputFileLuid0}.csv \
-q true \
-destLIMSID {compoundOutputFileLuid0} \
-l {compoundOutputFileLuid1}"
```

Routing Script Automation

Automatically triggered on exit of the step, this automation invokes the changeWorkflow script, which routes step outputs to the NovaSeq 6000 v3.3 workflow, and queues them for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.3) step.

Default automation command line is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar
-u {username} -p {password} -i {stepURI:v2} -l {compoundOutputFileLuid2}
script:changeWorkflow \
\
--FIELD_NAME 'N/A' \
--FIELD_VALUE 'N/A' \
--WORKFLOW 'NovaSeq 6000 v3.3' \
--STEP 'AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.3)' \
--INPUTS_OR_OUTPUTS 'OUTPUTS'"
```

Master Step Fields

There are 26 fields defined on the Dilute and Denature (NovaSeq 6000 v3.3) step. These fields are required for sample sheet and JSON file generation.

The following table lists field configuration details.

Table 162 Dilute and Denature (NovaSeq 6000 v3.3) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Analysis Software Version	Text	Required: No Read Only: No	Only applicable for v2 sample sheet. Otherwise, leave this field blank.
BaseSpace Sequence Hub Configuration	Text Dropdown	Required: Yes Read Only: No Custom Entries: No	Presets • Not Used • Run Monitoring Only • Run Monitoring and Storage
Custom Recipe Path	Text	Required: No Read Only: No	Not applicable
Experiment Name	Text	Required: Yes Read Only: No	Not applicable

Field Name	Field Type	Options	Additional Options and Dropdown Items
Index Read 1	Numeric Dropdown	Required: Yes Read Only: No Custom Entries: Yes	Range = 0–20 Decimal Places Displayed: 0 Presets • 0 • 6 • 8
Index Read 2	Numeric Dropdown	Required: Yes Read Only: No Custom Entries: Yes	Range = 0–20 Decimal Places Displayed: 0 Presets • 0 • 6 • 8
Output Folder	Text	Required: Yes Read Only: No	Not applicable
Override Cycles	Text	Required: No Read Only: No	Only applicable for v2 sample sheet. Otherwise, leave this field blank.
Paired End	Text Dropdown	Required: Yes Read Only: No Custom Entries: No	Presets • True • False
Read 1 Cycle	Numeric Dropdown	Required: Yes Read Only: No Custom Entries: Yes	Range = 0–251 Decimal Places Displayed: 0 Presets • 251* • 151 • 101 • 51 *Value of 251 is only supported for SP flow cell type. For all other cell types, maximum value is 151.

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Field Name	Field Type	Options	Additional Options and Dropdown Items
Read 2 Cycle	Numeric Dropdown	Required: Yes Read Only: No Custom Entries: Yes	Range = 1–251 Decimal Places Displayed: 0 Presets • 251* • 151 • 101 • 51 *Value of 251 is only supported for SP flow cell type. For all other cell types, maximum value is 151.
Reverse Complement Workflow	Toggle Switch	Required: Yes Read Only: No	Default • Yes
Run Mode (Not displayed in user interface)	Text Dropdown	Required: No Read Only: Yes Custom Entries: No	Presets • SP • S1 • S2 • S4
Samplesheet Format	Text Dropdown	Required: Yes Read Only: No Custom Entries: No	Presets • V1 (default) • V2
Setting Header (not displayed in user interface)	Text	Required: No Read Only: Yes	Not applicable
UMI—Read 1 Length	Numeric	Required: No Read Only: No Custom Entries: Yes	Range = 1
UMI—Read 1 Start From Cycle	Numeric	Required: No Read Only: No Custom Entries: Yes	Range = 1
UMI—Read 2 Length	Numeric	Required: No Read Only: No Custom Entries: Yes	Range = 1

Field Name	Field Type	Options	Additional Options and Dropdown Items
UMI—Read 2 Start From Cycle	Numeric	Required: No Read Only: No Custom Entries: Yes	Range = 1
Use Custom Index Read 1 Primer	Toggle Switch	Required: No Read Only: No	Default • None Set
Use Custom Read 1 Primer	Toggle Switch	Required: No Read Only: No	Default • None Set
Use Custom Read 2 Primer	Toggle Switch	Required: No Read Only: No	Default • None Set
Use Custom Recipe	Toggle Switch	Required: Yes Read Only: No	Default • None Set
Validation Script	Multiline Text	Required: Yes Read Only: Yes	Default value is provided in the drop-down section that follows the table. Do not remove this field as it is used by Validate Run Setup and Generate Sample Sheet automation script.
Workflow	Text	Required: No Read Only: Yes	Default • GenerateFASTQ
Workflow Type	Text Dropdown	Required: Yes Read Only: No Custom Entries: No	Presets • No Index • Single Index • Dual Index • Custom

Validation Script

step.::Run Mode:: = input.::Flowcell Type::; if (!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) { fail(::Experiment Name contains prohibited characters. Allowed characters are: a-z, A-Z, 0-9, -, and _::) }; if (step.::Workflow Type:: == ::No Index::) { if (step.::Index Read 1:: != 0 || step.::Index Read 2:: != 0) {

fail(::Index Read 1 and Index Read 2 must be 0 if No Index is selected.::) } } else if (step.::Workflow Type:: == ::Single Index::) { if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) { fail(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if Single Index is selected.::) } } else { if (step.::Index Read 1:: == 0 || step.::Index Read 2:: == 0) { fail(::Index Read 1 and Index Read 2 must be greater than 0 if Dual Index is selected.::) } }; if (step.::Paired End::.toBoolean()) { if (step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) { fail(::Read 1 Cycles and Read 2 Cycles must be greater than 0 if Paired End is True.::) } } else { if (step.::Read 2 Cycles:: != 0) { fail(::Read 2 Cycles must be 0 if Paired End is False.::) }; if (step.hasValue(::UMI - Read 2 Length::) || step.hasValue(::UMI - Read 2 Start From Cycle::)) { fail(::UMI - Read 2 Length and UMI - Read 2 Start From Cycle cannot be defined if Paired End is False.::) } }; if (input.::Flowcell Type:: != ::SP:: && step.::Read 1 Cycles:: > 151) { fail(::Read 1 Cycles must not be larger than 151 if it is not SPrime Flowcell::) }; if (input.::Flowcell Type:: != ::SP:: && step.::Read 2 Cycles:: > 151) { fail(::Read 2 Cycles must not be larger than 151 if it is not SPrime Flowcell::) }; if (step.hasValue(::UMI - Read 1 Length::) && !step.hasValue(::UMI - Read 1 Start From Cycle::)) { fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 1 Length is greater than 0.::) }; if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 1 Start From Cycle::)) { fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 1 Start From Cycle is greater than 0.::) }; if (step.hasValue(::UMI - Read 2 Length::) && !step.hasValue(::UMI - Read 2 Start From Cycle::)) { fail (::UMI - Read 2 Start From Cycle must be greater than 0 if UMI - Read 2 Length is greater than 0.::) }; if (!step.hasValue(::UMI - Read 2 Length::) && step.hasValue(::UMI - Read 2 Start From Cycle::)) { fail (::UMI - Read 2 Length must be greater than 0 if UMI - Read 2 Start From Cycle is greater than 0.::) }; if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 2 Length::)) { fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 2 Length is greater than 0.::) }; if (!step.hasValue(::UMI - Read 1 Start From Cycle::) && step.hasValue(::UMI - Read 2 Start From Cycle::)) { fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 2 Start From Cycle is greater than 0.::) }; step.::Settings Header:: = step.hasValue(::UMI - Read 1 Length::) || step.hasValue(::UMI - Read 2 Length::) ? ::[Settings]:: : :::::; nextStep = ::REMOVE::

Global Fields

The following table lists the global custom fields that are configured to display on the Dilute and Denature (NovaSeq 6000 v3.3) step.

Table 163 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			

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Field Name	Field Type	Options	Additional Options and Dropdown Items
Flowcell Type	Text Dropdown	Required: Yes Read Only: No Custom Entries: No	Presets • SP • S1 • S2 • S4
Loading Workflow Type	Text Dropdown	Required: Yes Read Only: No Custom Entries: No	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User Interaction

Refer to Run Dilute and Denature (NovaSeq 6000 v3.3) step section in *NovaSeq 6000 Integration v3.4.0 Validation and Troubleshooting* on page 596.

Protocol 3: NovaSeq Xp (NovaSeq 6000 v3.3)

Samples are routed to this protocol if their Loading Workflow Type value is set to NovaSeq Xp.

Samples are pooled and added to lanes on the NovaSeq flow cell type selected in the Define Run Format (NovaSeq 6000 v3.3) step. At the end of this protocol, the flow cell is sent to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.3) protocol.

This protocol contains the following three steps:

- Step 1: Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.3)
- Step 2: Dilute, Denature, and ExAmp (NovaSeq 6000 v3.3)
- Step 3: Load to Flowcell (NovaSeq 6000 v3.3)

Step 1: Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.3)

Manually place libraries into a pool.

Create only one pool per step.

Step input: NTP (normalized libraries)

Step output: Bulk pool

Validate Unique Indexes automation is not used. This functionality is handled by the core Clarity LIMS configuration for pooling.

Validate Inputs Flowcell Type and Single Pool Automation

Automatically triggered on exit of the Pooling screen, this automation completes the following actions:

- Checks that all samples in the pool have the same Flowcell Type assigned to them.
- Checks that only one pool has been created.
- Changes the value of error and logging messages to reference the type of pool passed as 'bulk' pool.

```
/opt/gls/clarity/bin/java \
-jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar \
script:validate_flowcell_for_input_pools \
-i {stepURI:v2} \
-u {username} \
-p {password} \
-l {compoundOutputFileLuid1} \
-validateSingleOutput true \
-poolType bulk"
```

Calculate Volumes Automation

Triggered when the Calculate Volumes button is selected on the Record Details screen, this automation completes the following actions:

- Calculates the number of samples in the pool.

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: + 1
```

- Sets the value of the Bulk Pool Volume (µl) and PhiX Volume (µl) field, based on the selected Flowcell Type.

```
step.::PhiX Volume (ul):: = !step.hasValue(::% PhiX (0.25nM) Spike-In::) ? 0 :
step.::% PhiX (0.25nM) Spike-In::;
if (input.::Flowcell Type:: == ::SP::) {
step.::Bulk Pool Volume (ul):: = step.'Number of Lanes to Sequence' * 18 + 30;
step.::PhiX Volume (ul):: *= 1.9;
};
if (input.::Flowcell Type:: == ::S1::) {
step.::Bulk Pool Volume (ul):: = step.'Number of Lanes to Sequence' * 18 + 30;
step.::PhiX Volume (ul):: *= 1.9;
};
if (input.::Flowcell Type:: == ::S2::) {
step.::Bulk Pool Volume (ul):: = step.'Number of Lanes to Sequence' * 22 + 30;
step.::PhiX Volume (ul):: *= 1.9;
};
```

```
if (input.::Flowcell Type:: == ::S4::) {
step.::Bulk Pool Volume (ul):: = step.'Number of Lanes to Sequence' * 30 + 30;
step.::PhiX Volume (ul):: *= 2.2;
};
if (step.::PhiX Volume (ul):: == 0) {
step.::PhiX Volume (ul):: = ::::;
};
```

- Calculates the Per Sample Volume (µl) to be added to the pool.

```
input.::Per Sample Volume (µl)::= (((input.::Final Loading Concentration (pM)::*
5/ 1000) / input.::Normalized Molarity (nM)::) * step.::Bulk Pool Volume (µl)::)
/ step.::Number of Samples in Pool::
```

> To ensure accurate pipetting of each sample in a pool for sequencing, the Per Sample Volume (µl) value must be equal to or higher than the Minimum Per Sample Volume (µl) (default value set at 5, may be edited).
>
> Assuming the default Minimum Per Sample Volume (µl) value of 5, completes the following steps for a given batch:
>
> 1. If the smallest Per Sample Volume (µl) value is less than 5, automatically assigns a value of 5 to the sample Adjusted Per Sample Volume (µl) field.
> 2. Adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

- Calculates the Total Sample Volume (µl) field value.

```
step.::Total Sample Volume (µl):: = step.::Total Sample Volume (µl):: +
input.::Adjusted Per Sample Volume (µl)::
```

- If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) field value.

```
if (step.::Total Sample Volume (µl):: >= step.::Bulk Pool Volume (µl)::)
{output.::RSB Volume (µl):: = 0} else {output.::RSB Volume (µl):: = step.::Bulk
Pool Volume (µl):: - step.::Total Sample Volume (µl)::} ;
```

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs.

```
output.::Flowcell Type::= input.::Flowcell Type::;output.::Loading Workflow
Type::= input.::Loading Workflow Type::;
```

- Uses the NovaSeq_Xp_Bulk_Pool.csv and NovaSeq_Xp_Bulk_Pool2.csv template files to generate a single CSV file containing information about the bulk pool and the samples it contains. The generated file is available for download on the Step Setup screen of the following step - Dilute, Denature, and ExAmp (NovaSeq 6000 v3.3).

```
&& /opt/gls/clarity/bin/java \-jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar \
```

```
  -i {stepURI:v2} \
  -u {username} \
  -p {password} \
  -l {compoundOutputFileLuid1} \ script:driver_file_generator \
  -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_Bulk_
Pool.csv \
  -o {compoundOutputFileLuid0}.csv \ script:driver_file_generator \
  -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_Bulk_
Pool2.csv \
  -o append.csv \
&& cat append.csv >> {compoundOutputFileLuid0}.csv"
```

- Resets the Total Sample Volume (ul) and Number of Samples in Pool field values so that the automation is idempotent.

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: - 1;
step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: -
input.::Adjusted Per Sample Volume (ul)::
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation completes the following actions:

- Copies the Flowcell Type values from the step inputs to the step outputs.
- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol.

```
output.::Flowcell Type:: = input.::Flowcell Type:: ; nextStep = ::ADVANCE::
```

Master Step Fields

There are seven fields defined on the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.3) step.

Table 164 Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.3) Master Step Field Configuration

Field Name	Field Type	Options	Additional Additional Options and Dropdown Items
% PhiX (0.25nM) Spike-in	Numeric	Required: No Read Only: No	Range = 0–100
Bulk Pool Volume (µl) (for calculation purposes, not displayed)	Numeric	Required: No Read Only: No	Decimal Places Displayed: 2

Field Name	Field Type	Options	Additional Additional Options and Dropdown Items
Minimum Per Sample Volume (uL)	Numeric	Required: Yes Read Only: No	Decimal Places Displayed: 2 Default • 5
Number of Lanes to Sequence	Numeric	Required: Yes Read Only: No	Decimal Places Displayed: 0
Number of Samples in Pool (for calculation purposes, not displayed)	Numeric	Required: No Read Only: No	Decimal Places Displayed: 0 Default • 0
PhiX Volume (ul) (for calculation purposes, not displayed)	Numeric	Required: No Read Only: No	Decimal Places Displayed: 2
Total Sample Volume (µl) (for calculation purposes, not displayed)	Numeric	Required: No Read Only: No	Decimal Places Displayed: 0 Default • 0

Global Fields

The following table lists the global custom fields that are configured to display on the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.3) step.

Table 165 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
RSB Volume (µl)	Numeric	Required: No Read Only: Yes	Decimal Places Displayed: 2

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Field Name	Field Type	Options	Additional Options and Dropdown Items
Flowcell Type	Text Dropdown	Required: Yes Read Only: No Custom Entries: No	Presets • SP • S1 • S2 • S4
Loading Workflow Type	Text Dropdown	Required: Yes Read Only: No Custom Entries: No	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User Interaction

Refer to Run Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.3) step section in *NovaSeq 6000 Integration v3.4.0 Validation and Troubleshooting* on page 596.

Step 2: Dilute, Denature and ExAmp (NovaSeq 6000 v3.3)

In this step, pooled samples are denatured and diluted by the addition of DPX, NaOH, Tris-HCl, and RSB. Manually create working pools based on the number of lanes you want to sequence.

Step input: Bulk pool

Step output: Working pool - variable number, choose how many working pools to create per bulk pool

Validate Inputs Flowcell Type Automation

Automatically triggered at the beginning of the step, this automation invokes the validate_flowcell_for_input_pools script. This script completes the following actions:

- Checks the inputs to the step and validates that the Flowcell Type field is set to a valid value (SP, S1, S2, or S4) and that all inputs have the same value for this field.
- The container type selected matches the value in the Flowcell Type field.

```
bash -l -c "/opt/gls/clarity/bin/java \
-jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar \
script:validate_flowcell_for_input_pools \
-i {stepURI:v2} \
-u {username} \
-p {password} \
-l {compoundOutputFileLuid1}"
```

Calculate Volumes Automation

Automatically triggered on entry to of the Record Details screen, this automation sets the value of the following fields based on the Flowcell Type:

- BP Aliquot Volume (µl)
- NaOH Volume (µl)
- Tris-HCl Volume (µl)
- DPX1 Volume (µl)
- DPX2 Volume (µl)
- DPX3 Volume (µl)
- Mastermix per Lane (µl)

```
if ( input.::Flowcell Type:: == ::SP:: ) {
output.::BP Aliquot Volume (µl):: = 18 ;
output.::NaOH Volume (µl):: = 4.50 ;
output.::Tris-HCl Volume (µl):: = 4.50 ;
step.::DPX1 Volume (µl):: = 126 ;
step.::DPX2 Volume (µl):: = 18 ;
step.::DPX3 Volume (µl):: = 66 ;
output.::Mastermix per Lane (µl):: = 63
};

if (input.::Flowcell Type:: == ::S1::) {
output.::BP Aliquot Volume (µl):: = 18;
output.::NaOH Volume (µl):: = 4.50;
output.::Tris-HCl Volume (µl):: = 4.50;
step.::DPX1 Volume (µl):: = 126;
step.::DPX2 Volume (µl):: = 18;
step.::DPX3 Volume (µl):: = 66;
output.::Mastermix per Lane (µl):: = 63
};
if (input.::Flowcell Type:: == ::S2::) {
output.::BP Aliquot Volume (µl):: = 22;
output.::NaOH Volume (µl):: = 5.50;
output.::Tris-HCl Volume (µl):: = 5.50;
step.::DPX1 Volume (µl):: = 126;
step.::DPX2 Volume (µl):: = 18;
step.::DPX3 Volume (µl):: = 66;
output.::Mastermix per Lane (µl):: = 77
```


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```
};
if (input.::Flowcell Type:: == ::S4::) {
output.::BP Aliquot Volume (µl):: = 30;
output.::NaOH Volume (µl):: = 7.50;
output.::Tris-HCl Volume (µl):: = 7.50;
step.::DPX1 Volume (µl):: = 315;
step.::DPX2 Volume (µl):: = 45;
step.::DPX3 Volume (µl):: = 165;
output.::Mastermix per Lane (µl):: = 105
};
```

The automation also completes the following actions:

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs.

```
output.::Flowcell Type::= input.::Flowcell Type::;
output.::Loading Workflow Type::= input.::Loading Workflow Type::;
```

- Uses the NovaSeq_Xp_Working_Pool.csv and NovaSeq_Xp_Working_Pool2.csv template files to generate a single CSV file containing information about the DPX volume and the volume of BP Aliquot, Mastermix, NaOH, and Tris-HCl to add per working pool. The generated file is available for download on the Step Setup screen of the following step - Load to Flowcell (NovaSeq 6000 v3.3).

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_Working_
Pool.csv \
-o {compoundOutputFileLuid0}.csv \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_Working_
Pool2.csv \
-o append.csv \
&& cat append.csv >> {compoundOutputFileLuid0}.csv"
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation:

- Copies the Flowcell Type values from the step inputs to the step outputs.
- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ; nextStep = ::ADVANCE::
```

Master Step Fields

There are three fields defined on the Dilute, Denature, and ExAmp (NovaSeq 6000 v3.3) step. These field values are set by a script and are not editable while running the step.

The following table lists field configuration details.

Table 166 Dilute, Denature, and ExAmp (NovaSeq v3.3) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
DPX1 Volume (µl)	Numeric	Required: No Read Only: Yes	Decimal Places Displayed: 0
DPX2 Volume (µl)	Numeric	Required: No Read Only: Yes	Decimal Places Displayed: 0
DPX3 Volume (µl)	Numeric	Required: No Read Only: Yes	Decimal Places Displayed: 0

Global Fields

The following table lists the global custom fields that are configured to display on the Dilute, Denature, and ExAmp (NovaSeq 6000 v3.3) step.

Table 167 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
BP Aliquot Volume (µl)	Numeric	Required: No Read Only: Yes	Decimal Places Displayed: 0
Flowcell Type	Text Dropdown	Required: Yes Read Only: No Custom Entries: No	Presets • SP • S1 • S2 • S4

Field Name	Field Type	Options	Additional Options and Dropdown Items
Loading Workflow Type	Text Dropdown	Required: Yes Read Only: No Custom Entries: No	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
Mastermix per Lane (µl)	Numeric	Required: No Read Only: Yes	Decimal Places Displayed: 0
NaOH Volume (µl)	Numeric	Required: No Read Only: Yes	Decimal Places Displayed: 2
Tris-HCl Volume (µl)	Numeric	Required: No Read Only: Yes	Decimal Places Displayed: 2

User Interaction

Refer to Run Dilute, Denature & ExAmp (NovaSeq 6000 v3.3) step section in *NovaSeq 6000 Integration v3.4.0 Validation and Troubleshooting* on page 596.

Step 3: Load to Flowcell (NovaSeq 6000 v3.3)

In this step, scan the flow cell barcode into the Clarity LIMS and manually place the working pools into the lanes of the flow cell for the NovaSeq run. This step validates the run setup information and generates the sample sheet file.

Step input: Working pool

Step output: Flow cell (output containers: SP, S1, and S2 with 2 lanes, and S4 with 4 lanes)

Validate Inputs and Selected Container Automation

Automatically triggered at the beginning of the step, this automation invokes the validate_flowcell_for_input_pools and validateSelectedContainer scripts. These scripts validate the step inputs and the container selected by the user, as follows.

- Checks that the Flowcell Type field has been set to a valid value (SP, S1, S2, or S4) and that all inputs have the same value.
- Checks that the number of outputs matches the number of lanes on the selected flow cell type. If validation fails, an error message informs that the number of working pools does not match the number of lanes available on the flow cell.
  The following table defines the number of samples allowed:

Flow Cell Type	Number of Working Pools
SP	2
S1	2
S2	2
S4	4

- Checks that the container type selected on entry to the Placement screen matches the value in the Flowcell Type field. If validation fails, an error message displays.

```
bash -l -c "/opt/gls/clarity/bin/java \
-jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar
\script:validate_flowcell_for_input_pools \
  -i {stepURI:v2} \
  -u {username} \
  -p {password} \
  -l {compoundOutputFileLuid1} \
  -validateSelectedContainer true"
```

Validate Flowcell Barcode Automation

Automatically triggered on exit of the Placement screen, this automation validates the flow cell barcode scanned into the Clarity LIMS by the user, using the following logic:

```
if (input.::Flowcell Type:: == ::SP:: && !output.container.name.matches( ::[A-
Za-z0-9]{5}DRX[XY2357]:: ) ) {
fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: )
};
if (input.::Flowcell Type:: == ::S1:: && !output.container.name.matches( ::[A-
Za-z0-9]{5}DRX[XY2357]:: ) ) {
fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: )
};
if (input.::Flowcell Type:: == ::S2:: && !output.container.name.matches( ::[A-
Za-z0-9]{5}DMX[XY2357]:: ) ) {
fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: )
};
if (input.::Flowcell Type:: == ::S4:: && !output.container.name.matches( ::[A-
Za-z0-9]{5}DSX[XY2357]:: ) ) {
fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: )
};
```

- Copies the Flowcell Type and Loading Workflow Type field values from step inputs to outputs:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ; \
```

```
output.::Loading Workflow Type:: = input.::Loading Workflow Type::'
```

Validate Run Setup and Generate Sample Sheet Automation

The script definition portion of Validate Run Setup has been moved into the Validation Script custom field due to the 4000 character limitation.

- If modification is required to the Validate Run Setup script, perform the changes directly on Validation Script custom field default value.
- The script definition portion of Generate Sample Sheet is unchanged.

Automatically triggered by selecting a button on the Record Details screen, this automation:

- Copies the Flowcell Type from the step input to the Run Mode field (hidden):

```
step.::Run Mode:: = input.::Flowcell Type::
```

- Checks the Flowcell field value.
  - If Flowcell Type value is not SP, checks that the value of both Read 1 Cycles and Read 2 Cycles is 151 or less. If value is greater than 151, displays an error message.

```
if (input.::Flowcell Type::!=::SP:: && step.::Read 1 Cycles:: > 151) {
fail(::Read 1 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
}
if (input.::Flowcell Type::!=::SP:: && step.::Read 2 Cycles:: > 151) {
fail(::Read 2 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
}
```

- Validates the parameters entered on the Record Details screen, which are used to set up the run and generate the sample sheet file.
  - Experiment Name may only contain alphanumeric, dash, or underscore characters. Spaces are not permitted.
  - When Workflow Type = No Index, both Index Read 1 and Index Read 2 must be zero.
  - When Workflow Type = Single Index, Index Read 1 must be greater than zero and Index Read 2 must be zero.
  - When Workflow Type = Dual Index, both Index Read 1 and Index Read 2 must be greater than zero.

```
if(!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) {
fail(::Experiment Name contains prohibited characters. Allowed characters are:
a-z, A-Z, 0-9, -, and _::)
};
if (step.::Workflow Type:: == ::No Index::) {
if (step.::Index Read 1:: != 0 || step.::Index Read 2:: != 0) {
fail(::Index Read 1 and Index Read 2 must be 0 if No Index is selected.::)
```


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```
}
} else if (step.::Workflow Type:: == ::Single Index::) {
if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) {
fail(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if Single
Index is selected.::)
}
} else {
if (step.::Index Read 1:: == 0 || step.::Index Read 2:: == 0) {
fail(::Index Read 1 and Index Read 2 must be greater than 0 if Dual Index is
selected.::)
}
};
```

- Checks the Paired End and Read 2 Cycles field values.
  - If Paired End = True and Read 2 Cycles value is 0, an error displays.
  - If Paired End = False, Read 2 Cycles must be zero and UMI - Read 2 Length and UMI - Read 2 Start From Cycle must be empty.

```
if (step.::Paired End::.toBoolean()) {
if (step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) {
fail(::Read 1 Cycles and Read 2 Cycles must be greater than 0 if Paired End is
True.::)
}
} else {
if (step.::Read 2 Cycles:: != 0) {
fail(::Read 2 Cycles must be 0 if Paired End is False.::)
};
if (step.hasValue(::UMI - Read 2 Length::) || step.hasValue(::UMI - Read 2 Start
From Cycle::)) {
fail(::UMI - Read 2 Length and UMI - Read 2 Start From Cycle cannot be defined
if Paired End is False.::)
}
};
```

- Checks the Flowcell field value.
  If Flowcell Type value is not SP, checks that the value of both Read 1 Cycles and Read 2 Cycles is 151 or less. If value is greater than 151, generates an error message.

```
if (input.::Flowcell Type::!=::SP:: && step.::Read 1 Cycles:: > 151) {
fail(::Read 1 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
}
if (input.::Flowcell Type::!=::SP:: && step.::Read 2 Cycles:: > 151) {
fail(::Read 2 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
```


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```
}
```

- Validates UMI Settings:
  - If UMI - Read 1 Length has value, UMI - Read 1 Start From Cycle must have value.
  - If UMI - Read 1 Length has no value, UMI - Read 1 Start From Cycle must not have value.
  - If UMI - Read 2 Length has value, UMI - Read 2 Start From Cycle must have value.
  - If UMI - Read 2 Length has no value, UMI - Read 2 Start From Cycle must not have value.
  - If UMI - Read 1 Length has not value, UMI - Read 2 Length must not have value.
  - If UMI - Read 1 Start From Cycle has no value, UMI - Read 2 Start From Cycle must not have value.

```
if (step.hasValue(::UMI - Read 1 Length::) && !step.hasValue(::UMI - Read 1
Start From Cycle::)) {
fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 1
Length is greater than 0.::)
};
if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 1
Start From Cycle::)) {
fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 1 Start From
Cycle is greater than 0.::)
};
if (step.hasValue(::UMI - Read 2 Length::) && !step.hasValue(::UMI - Read 2
Start From Cycle::)) {
fail(::UMI - Read 2 Start From Cycle must be greater than 0 if UMI - Read 2
Length is greater than 0.::)
};
if (!step.hasValue(::UMI - Read 2 Length::) && step.hasValue(::UMI - Read 2
Start From Cycle::)) {
fail(::UMI - Read 2 Length must be greater than 0 if UMI - Read 2 Start From
Cycle is greater than 0.::)
};
if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 2
Length::)) {
fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 2 Length is
greater than 0.::)
};
if (!step.hasValue(::UMI - Read 1 Start From Cycle::) && step.hasValue(::UMI -
Read 2 Start From Cycle::)) {
fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 2
Start From Cycle is greater than 0.::)
};
```


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- Validate Analysis Software Version

```
if (step.hasValue(::Analysis Software Version::) && !step.::Analysis Software
Version::.matches(::^(\\d+)(\\.\\d+)*$::)) {
fail(::Analysis Software Version contains prohibited characters. Allowed
characters are 0-9 and period. It shall start and end with numbers and separated
by single period e.g. 3.8.4::)
};
```

- Checks Override Cycles field value.

```
if (step.hasValue(::Override Cycles::) && !step.::Override Cycles::.matches(::
[YNIU0-9;]+::)) {
fail(::Override Cycles contains prohibited characters. Allowed characters are:
Y, N, I, U, 0-9 and ;. Example: N1Y150;I8;I7N1;Y141U10.::)
};
```

- Outputs the Settings Header in sample sheet if UMI - Read 1 Length or UMI - Read 2 Length have value.

```
step.::Settings Header:: = step.hasValue(::UMI - Read 1 Length::) ||
step.hasValue(::UMI - Read 2 Length::) ? ::[Settings]:: : ::::;
```

- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol - AUTOMATED - Run (NovaSeq 6000) v3.3.

```
nextStep = ::ADVANCE::
```

- Generates the sample sheet and attaches it to the step. (Refer to *Sample Sheet Generation* on page 582 for more information.) The sample sheet template depends on the selected value for Samplesheet Format and/or Reverse Complete Workflow.

```
&& if [ '{udf:Samplesheet Format}' == 'V2' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Samplesheet_v2.csv'; \
elif [ '{udf:Reverse Complement Workflow}' == 'true' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Reverse_Complement_Samplesheet.csv'; \
elif [ '{udf:Reverse Complement Workflow}' == 'false' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Samplesheet.csv'; \
else echo 'UDF Reverse Complement Workflow is empty or undefined.'; exit -1; fi
\
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/$templateFilename \
-o {compoundOutputFileLuid0}.csv \
-q true \
-destLIMSID {compoundOutputFileLuid0} \
```

```
-l {compoundOutputFileLuid1}"
```

Master Step Fields

There are 26 fields defined on the Load to Flowcell (NovaSeq 6000 v3.3) step.

The following table lists the field configuration details.

Table 168 Load to Flowcell (NovaSeq 6000 v3.3) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Analysis Software Version	Text	Required: No Read Only: No	Only applicable for v2 sample sheet. Otherwise, leave this field blank.
BaseSpace Sequence Hub Configuration	Text Dropdown	Required: Yes Read Only: No Custom Entries: No	Presets • Not Used • Run Monitoring Only • Run Monitoring and Storage
Custom Recipe Path	Text	Required: No Read Only: No	Not applicable
Experiment Name	Text	Required: Yes Read Only: No	Not applicable
Index Read 1	Numeric Dropdown	Required: Yes Read Only: No Custom Entries: Yes	Range = 0–20 Decimal Places Displayed: 0 Presets • 0 • 6 • 8
Index Read 2	Numeric Dropdown	Required: Yes Read Only: No Custom Entries: Yes	Range = 0–20 Decimal Places Displayed: 0 Presets • 0 • 6 • 8
Library Tube Barcode	Text	Required: Yes Read Only: No	Not applicable
Output Folder	Text	Required: Yes Read Only: No	Not applicable

Field Name	Field Type	Options	Additional Options and Dropdown Items
Override Cycles	Text	Required: No Read Only: No	Only applicable for v2 sample sheet. Otherwise, leave this field blank.
Paired End	Text Dropdown	Required: Yes Read Only: No Custom Entries: No	Presets • True • False
Read 1 Cycles	Numeric Dropdown	Required: Yes Read Only: No Custom Entries: Yes	Range = 0–251 Decimal Places Displayed: 0 Presets • 251* • 151 • 101 • 51 *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151
Read 2 Cycles	Numeric Dropdown	Required: Yes Read Only: No Custom Entries: Yes	Range = 0–251 Decimal Places Displayed: 0 Presets • 251* • 151 • 101 • 51 *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151
Reverse Complement Workflow	Toggle Switch	Required: Yes Read Only: No	Default • Yes
Run Mode (not displayed in user interface)	Text Dropdown	Required: No Read Only: Yes Custom Entries: No	Presets • SP • S1 • S2 • S4

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Field Name	Field Type	Options	Additional Options and Dropdown Items
Samplesheet Format	Text Dropdown	Required: No Read Only: No Custom Entries: No	Presets • V1 (default) • V2
Settings Header (not displayed in user interface)	Text	Required: No Read Only: Yes	Not applicable
UMI—Read 1 Length	Numeric	Required: No Read Only: No Custom Entries: Yes	Range = 1
UMI—Read 1 Start From Cycle	Numeric	Required: No Read only: No Custom Entries: Yes	Range = 1
UMI—Read 2 Length	Numeric	Required: No Read only: No Custom Entries: Yes	Range = 1
UMI—Read 2 Start From Cycle	Numeric	Required: No Read only: No Custom Entries: Yes	Range = 1
Use Custom Index Read 1 Primer	Toggle Switch	Required: No Read Only: No	Default • None Set
Use Custom Read 1 Primer	Toggle Switch	Required: No Read Only: No	Default • None Set
Use Custom Read 2 Primer	Toggle Switch	Required: No Read Only: No	Default • None Set
Use Custom Recipe	Toggle Switch	Required: Yes Read Only: No	Default • No
Validation Script	Multiline Text	Required: Yes Read Only: Yes	Default value provided in the drop-down section following the table. Do not remove this field as it is used by Validate Run Setup and Generate Sample Sheet automation script.

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Field Name	Field Type	Options	Additional Options and Dropdown Items
Workflow	Text	Required: No Read Only: Yes	Default • GenerateFASTQ
Workflow Type	Text Dropdown	Required: Yes Read Only: No Custom Entries: No	Presets • No Index • Single Index • Dual Index • Custom

Validation Script

step.::Run Mode:: = input.::Flowcell Type::; if (!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) { fail(::Experiment Name contains prohibited characters. Allowed characters are: a-z, A-Z, 0-9, -, and _::) }; if (step.::Workflow Type:: == ::No Index::) { if (step.::Index Read 1:: != 0 || step.::Index Read 2:: != 0) { fail(::Index Read 1 and Index Read 2 must be 0 if No Index is selected.::) } } else if (step.::Workflow Type:: == ::Single Index::) { if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) { fail(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if Single Index is selected.::) } } else { if (step.::Index Read 1:: == 0 || step.::Index Read 2:: == 0) { fail(::Index Read 1 and Index Read 2 must be greater than 0 if Dual Index is selected.::) } }; if (step.::Paired End::.toBoolean()) { if (step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) { fail(::Read 1 Cycles and Read 2 Cycles must be greater than 0 if Paired End is True.::) } } else { if (step.::Read 2 Cycles:: != 0) { fail(::Read 2 Cycles must be 0 if Paired End is False.::) }; if (step.hasValue(::UMI - Read 2 Length::) || step.hasValue(::UMI - Read 2 Start From Cycle::)) { fail(::UMI - Read 2 Length and UMI - Read 2 Start From Cycle cannot be defined if Paired End is False.::) } }; if (input.::Flowcell Type:: != ::SP:: && step.::Read 1 Cycles:: > 151) { fail(::Read 1 Cycles must not be larger than 151 if it is not SPrime Flowcell::) }; if (input.::Flowcell Type:: != ::SP:: && step.::Read 2 Cycles:: > 151) { fail(::Read 2 Cycles must not be larger than 151 if it is not SPrime Flowcell::) }; if (step.hasValue(::UMI - Read 1 Length::) && !step.hasValue(::UMI - Read 1 Start From Cycle::)) { fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 1 Length is greater than 0.::) }; if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 1 Start From Cycle::)) { fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 1 Start From Cycle is greater than 0.::) }; if (step.hasValue(::UMI - Read 2 Length::) && !step.hasValue(::UMI - Read 2 Start From Cycle::)) { fail (::UMI - Read 2 Start From Cycle must be greater than 0 if UMI - Read 2 Length is greater than 0.::) }; if (!step.hasValue(::UMI - Read 2 Length::) && step.hasValue(::UMI - Read 2 Start From Cycle::)) { fail (::UMI - Read 2 Length must be greater than 0 if UMI - Read 2 Start From Cycle is greater than 0.::) }; if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 2 Length::)) { fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 2 Length is greater than 0.::) }; if (!step.hasValue(::UMI - Read 1 Start From Cycle::) && step.hasValue(::UMI - Read 2 Start From Cycle::)) { fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 2 Start From Cycle is greater than 0.::) }; step.::Settings Header:: = step.hasValue(::UMI - Read 1 Length::) || step.hasValue(::UMI - Read 2 Length::) ? ::[Settings]:: : :::::; nextStep = ::ADVANCE::

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Global Fields

The following table lists the global custom fields that are configured to display on the Load to Flowcell (NovaSeq 6000 v3.3) step.

Table 169 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
Flowcell Type	Text Dropdown	Required: Yes Read Only: No Custom Entries: No	Presets • SP • S1 • S2 • S4
Loading Workflow Type	Text Dropdown	Required: Yes Read Only: No Custom Entries: No	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User Interaction

Refer to Run Load to Flowcell (NovaSeq 6000 v3.3) step section in *NovaSeq 6000 Integration v3.4.0 Configuration and User Interaction* on page 536.

Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.3)

This final protocol contains one fully automated step, AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.3), described in the following section.

> This is a fully automated step. Do not add samples to the Ice Bucket or start the step manually. The sequencing service does not update samples correctly if they have been manually started.

Step 1: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.3)

Step input: Library tube from NovaSeq Standard or flow cell from NovaSeq Xp protocol

Step output: Result file/measurement

In this step, pooled samples are sequenced on the NovaSeq instrument and the run metrics are recorded in Clarity LIMS.

Set Next Steps Automation

By default, the Set Next Steps automation is disabled (set to Not Used) and the Sequencer API sets the next step for samples.

If necessary, override the default next step behavior used by the Sequencer API by performing the following steps:

1. Enable the automation and use it to set custom next step behavior and generate a log file. By default, the automation sets the next step to ADVANCE and the samples complete the protocol.
2. Configure the automation to trigger automatically on exit of the Record Details screen.
3. Disable or configure auto-complete behavior by setting the values of the run.autoComplete and autoCompleteOnlyAtSuccess properties. For details, see *Configuring Autocomplete Properties* on page 594.

Update Lane Number Automation

This automation triggers automatically upon entry to the Record Details screen.

Do not disable or modify this automation to ensure the Lane Number displays properly.

Master Step Fields

The following table lists the run parameters parsed from the RunParameter.xml file and mapped to fields configured on the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.3) step.

Table 170 Run Parameters - Field Mappings

Step UDF / Custom Field Name
Current Cycle
Current Read
Firmware Version
Flow Cell Expiration Date
Flow Cell ID
Flow Cell Lot Number
Flow Cell Mode
Flow Cell Part Number
Flow Cell Side
Instrument Control Software Version
Instrument ID
Instrument Type
Lane Counter
Loading Workflow Type

Step UDF / Custom Field Name
Output Folder
RTA Version
Run Completion Date
Run ID
Run Status
Sequencing Log

Global Fields

The following global custom fields are used to capture the run metrics in Clarity LIMS.

- % Aligned R1
- % Aligned R2
- % Bases >=Q30 R1
- % Bases >=Q30 R2
- % Error Rate R1
- % Error Rate R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- %PF R1
- %PF R2
- Cluster Density (K/mm^2) R1
- Cluster Density (K/mm^2) R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- Reads PF (M) R1
- Reads PF (M) R2
- Yield PF (Gb) R1
- Yield PF (Gb) R2

Note the following details:

- Values are aggregated across all lanes. Some values, for example Yield PF (Gb) R1, are summed while others are averaged.
- The names listed previously are the default global custom field names installed with the NovaSeq Integration v3.4.0 configuration provided in the Illumina Preset Protocols (IPP) v2.3 or later.
- All global configuration fields are configured on the Container entity.
- All field names are configurable via the Clarity LIMS Configuration > Custom Fields screen (Global Fields tab).
- If field names are changed in Clarity LIMS, they must also be changed through the run.metricUdfNames properties file in the application.yml file for the Sequencer API (see *application.yml Properties* on page 589. All fields are configured to be visible on the Record Details screen for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.3) step (in the Sample Details table).

These global custom fields are configured on the Container entity, visible on each input, but they should not be confused with per-lane metrics.

All run metrics tracked in Clarity LIMS are overall metrics for the run. There are currently no per-lane metrics provided by NovaSeq Control Software.

Sample Sheet Generation

The sample sheet is generated on the step before the run, which is Dilute and Denature (NovaSeq 6000 v3.3) in the NovaSeq Standard protocol or Load to Flowcell (NovaSeq 6000 v3.3) in the NovaSeq Xp protocol. This step places samples on the library tube or flow cell that are loaded in the NovaSeq instrument.

In the default configuration, the Validate Run Setup and Generate Sample Sheet automation generates one CSV format sample sheet file for use with bcl2fastq v2.20 downstream analysis.

The sample sheet is uploaded to the NVCS via the /Illumina/Sequencer/v2/sequencing-run/files endpoint to the Sequencer API. The file endpoint allows for a file to be downloaded from the Clarity LIMS using OAuth (instead of Basic Authentication), which is required for NovaSeq.

The run recipe response sets the sample sheet URL to the link to download the file from this endpoint and sets the sampleSheetRequiresOAuth value to true.

Sample Sheet Content

The sample sheet content is controlled by the following step UDFs/custom fields that display in the Step Details area of the Record Details screen.

- Experiment Name
- Read 1 Cycles
- Read 2 Cycles
- Workflow

- Reverse Complement Workflow
- Samplesheet Format

  Depending on Samplesheet Format field value selection, a v1 or v2 sample sheet is generated.

  See the Bcl2fastq2 Sample Sheet Generation section of *Illumina Instrument Sample Sheets (NGS v5.17 & later)* on page 2542 for detailed information on the following items:
- Sample sheet generation script parameters and usage
- Sample sheet data and configuration options
- Enabling unique FASTQ file names per sequencing run

NovaSeq Run

The following steps outline the sequence of events that occurs when a flow cell is loaded onto the NovaSeq instrument.

User Authentication and Login

User authentication and login to the NovaSeq instrument is achieved via the getSequencerLoginURL endpoint (found at /Illumina/Sequencer/v2/sequencing-run/login). This endpoint returns the URL that the instrument uses to get an access token.

1. The Sequencer API service provides the NovaSeq Control Software (NVCS) with a URL for the Clarity LIMS login screen and the NVCS displays this screen to the user. Log in to automatically authorize the NVCS to use your account to access the Clarity LIMS.
2. Enter credentials:
   - Username and password are passed to the Clarity LIMS server to make sure that the access is valid.
   - By default, the Sequencer API service tries to contact the Clarity LIMS at http://localhost:9080/clarity/. This URL is set by the installation script, but it can be configured with the clarity.url property in the application.yml file (see *application.yml Properties* on page 589
3. The user is redirected to the Sequencer login page at /Illumina/Sequencer/v2/sequencer_login.
   - If authentication is successful, the screen displays a success message. The Clarity LIMS server provides the OAuth token and username by returning them in redirect to /Illumina/Sequencer/v2/sequencer_login/Success. This redirect does not reload the page, but allows the NovaSeq to continue with run setup.
   - If authentication fails, the user is left on the login screen and a warning message informs them of the failure. A background redirect to /Illumina/Sequencer/v2/sequencer_login/Error informs the NovaSeq of the failure.

Respond to Validation / Recipe Request Call from Instrument

1. The sequencing-run/recipe/novaseq endpoint consumes a POST request with a LibraryContainerI, FlowCellId, and reagents information.
   - LibraryContainerId, FlowCellId, and reagent information is provided by the NovaSeq.
   - LibraryContainerId and FlowCellId should match either the library tube ID or flow cell ID of the corresponding containers in Clarity LIMS.
   - If there are no containers or multiple containers, an error is generated.
2. All populated containers whose name matches either the LibraryContainerId or FlowCellId are validated as follows.
   - All samples in the container must be queued for a sequencing run step.
   - All samples must be queued for only one sequencing run step.
   - All samples must be queued for the same sequencing run step.

   Configure the steps that are considered sequencing run steps in the novaseq.sequenceStepNames property in the application.yml file. The property is a list and each list entry must be the exact name of a sequencing step, not the name of the underlying master step/process type. See *application.yml Properties* on page 589 for more details.
3. After a single container has passed validation, the run setup information (run recipe request) is returned to the NVCS via the API and the run recipe is generated.
   Note the following details:
   - The run recipe information is read from fields configured on the parent process/master step of the samples in the container. The workflow must include recipe definition in that step.
   - By default, the parent process/master step is the Dilute and Denature (NovaSeq 6000 v3.3) or Load to Flowcell (NovaSeq 6000 v3.3) step, depending on workflow.
   - The default field values match the names of the fields in the NovaSeq v3.4.0 configuration included in IPP. The field names are configurable through the recipe.udfNames.* properties. Each of these properties corresponds to an entry in the recipe and the value of a property defines the name of the field that is used to populate that entry in the recipe.
   - It is assumed that this parent process/master step removes any illegal characters from the Experiment Name field.
   - The Clarity LIMS download link to the sample sheet created on the run setup step is used for the SampleSheet value in the recipe. The name of the output of the sample sheet on the setup step can be configured through the recipe.sampleSheet.outputName property (Sample Sheet by default) in the application.yml (see *application.yml Properties* on page 589 for more details). If no matching output is found, or no file was attached to the output, then the value of the recipe.sampleSheet.notAvailableValue property (Not Available by default) is used in the recipe.

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4. After the recipe is generated, it is returned as a *.json file in the response, in the form defined by the Swagger definition for the API.

Run Recipe Contents

The contents of the run recipe are controlled by the sample sheet contents and the fields configured on the Dilute and Denature (NovaSeq 6000 v3.3) and Load to Flowcell (NovaSeq 6000 v3.3) steps:

Example of run recipe content is as follows:

```
{
  "run_name": "NVCS16027_SPrime_2x250",
  "run_mode": "S4",
  "workflow_type": "DualIndex",
  "librarytube_id": "NV0025867-LIB",
  "flowcell_id": "",
  "sample_loading_type": "NovaSeqStandard",
  "rehyb": false,
  "paired_end": true,
  "read1": 151,
  "read2": 151,
  "index_read1": 8,
  "index_read2": 8,
  "output_folder": "\\\\network_path\\run_data",
  "samplesheet": "<path to sample sheet on LIMS server>",
  "require_samplesheet_authentication": true,
  "usecustomrecipe": false,
  "customRecipe": null,
  "use_basespace": false,
  "basespace_mode": null,
  "use_custom_read1_primer": false,
  "use_custom_read2_primer": false,
  "use_custom_index_read1_primer": false
}
```

Respond to Sequencing Started Call from Instrument

1. Matching container for the run (library tube or flow cell) is found, and contents are validated to be queued for the correct step.

   If no matching container is found or if the samples in the container are incorrectly queued, an error is thrown and the step is not started. This validation is also done during the run recipe request immediately prior, so this error case should not be encountered.

2. Reagent kits that are configured for the Sequencer API are checked against the configuration for the step to be run:
   - If a kit is configured for the API but does not exist in Clarity LIMS, it is created.
   - If a kit exists, but is not enabled on the step to be run, the step configuration is updated to enable it.
     - If a kit that is to be enabled is Archived, it is set to Active.
     - If a kit that is to be enabled is in Pending status, it is set to Active.
   - If any kits enabled on the step are not configured for the Sequencer API, a warning is logged and the step cannot be completed by the API without user intervention.
   - If any kits provided in the recipe request are not configured for the Sequencer API, they are ignored and a warning is logged during lot tracking.
3. The number of replicates for the step is determined by the type of flow cell being used in the run.
   - For Standard mode, this information is based on the number of lanes in the flow cell. This information is stored as part of the Sequencer API configuration.
   - For XP mode, there is always one replicate, because there is already one input tracked for each lane.
4. The automated sequencing step is started.
5. Reagent lots are attached to the step. The Flow Cell and Library Tube reagents in the request are ignored. Information from these reagents is tracked in Clarity LIMS as containers and custom fields. Any other reagents in the request are handled as follows.
   - If the reagent is not configured to be tracked, a warning is logged and the reagent is ignored.
   - Existing reagent lots are looked up by kit name (determined by Sequencer API configuration), name (the serial number in the request), and lot number (the lot number in the request).
   - If one or more matching reagent lots exist in Clarity LIMS, the newest one is used.
   - If the expiry date does not match the expiry in the request, or if the status is not set to Active, the lot is updated to have the correct expiry date and an Active status.

   Otherwise a new lot is created with the following configuration:
   - Reagent Kit—Matching kit as determined from Sequencer API configuration and step 2.
   - Name—Serial number in request.
   - Lot Number—Lot number in request.
   - Expiry—Expiry date in request.
   - Status—Active.

   If any reagents are expected to be in the request but are not, these reagents are logged. This action prevents the step from being completed without user intervention.

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6. Master step custom fields are updated with the run information parsed from the request. See *Respond to Record InterOps Metrics Call from Instrument* on page 587.
7. Any important events during this request are logged into the Sequencer API log file and in the Sequencing Log multiline global custom field on the step (assuming the step was started successfully). See *Respond to Record InterOps Metrics Call from Instrument* on page 587.

Respond to Record InterOps Metrics Call from Instrument

When the metrics endpoint receives a POST, it completes the following actions:

- Looks for a matching library tube or flow cell in Clarity LIMS.
- Find the in-progress sequencing step for that container.

When the step is found, the run metrics in the request are written to container global custom fields in Clarity LIMS.

Respond to Sequencing Completed Call from Instrument

If the sequencing run is successful, after the metrics are recorded, the API call for Sequencing Completed is received. Information is matched by Sequencing Container and the following occurs in Clarity LIMS:

1. The Current Cycle, Current Read, and Status master step custom fields are updated.
2. The multiline Sequencing Log master step field is updated.
3. A success message is recorded in the log file at the info level:

```
Sequencing run with Run ID {runId} of Step '{step.name}' ({step.limsid})
completed successfully.
```

4. The Clarity LIMS waits for any automations that are triggered automatically as it advances the step.
5. By default, the Clarity LIMS assigns the next steps on the outputs to move them on to the first configured next step. If there are no next steps, the protocol is completed.

Respond to Sequencing Errored Call from Instrument

If the sequencing run is unsuccessful, the API call for Sequencing Error is received. Information is matched by Sequencing Container and the following occurs in Clarity LIMS:

1. The Current Cycle, Current Read, and Status master step custom fields are updated.
2. The multiline Sequencing Log master step field is updated.
3. A failure message is recorded in the log file at the error level:

```
Step '${Step.name}' (${Step.limsid}) failed with Run ID ${runId}.
```

4. The step is left at the Record Details screen.

Respond to Sequencing RunEndedByUser Call from Sequencer

If the sequencing run is ended by the user, the API call for Sequencing RunEndedByUser is received. Information is matched by Sequencing Container and the following occurs in Clarity LIMS:

1. The Current Cycle, Current Read, and Status master fields are updated.
2. The multiline Sequencing Log master step field is updated.
3. The following message is recorded in the log file, at the warning level:

```
"Sequencing run with Run ID ${status.runInfo.runId} of Step
'${step.configuration.name}' (${step.limsid}) aborted by user
${status.runInfo.userName}."
```

4. The step is left at the Record Details screen.

Components Installed

The following sections describe the various components (files, properties, reagent categories / label groups, reagent kits, and containers) that are installed by default as part of this integration.

Illumina NovaSeq Integration v3.4.0 is distributed as an RPM package, BaseSpaceLIMS-sequencer-api. This RPM package must be installed on the Clarity LIMS server.

The BaseSpaceLIMS-sequencer-api RPM installs the following items:

- Sequencer API WAR file
- Configuration file - application.yml
- Two configuration scripts: configure_sequencer_api_proxy.sh and configure_sequencer_api_application.sh

The NovaSeq v3 configuration is delivered through the Illumina Preset Protocols (IPP) v2.3 which requires installation of the NGS Extensions Package v5.21.0 or later.

- If the NGS Extensions Package is not already installed, or if a version earlier than v5.21.0 is installed, the latest version is installed by default with the NovaSeq integration. See *Release Notes* for details.
- If NGS Extensions Package v5.21.0 or later is already installed, upgrade is not forced or required.

NovaSeq 6000 Integration RPM Components

The following table lists the components installed by the RPM package.

Files Installed	Location and Description
Illumina#Sequencer#v2.war	/opt/gls/clarity/tomcat/current/webapps War file for NovaSeq v3.4.0 API.

Files Installed	Location and Description
configure_sequencer_api_application.sh	/opt/gls/clarity/config/ Script that configures the Sequencer API application through its external application.yml file.
configure_sequencer_api_env.sh	/opt/gls/clarity/config/ Script that configures the Clarity LIMSTomcat configuration to include Secret Util settings.
configure_sequencer_api_proxy.sh	/opt/gls/clarity/config/ Script that configures the proxy to allow communication with the Sequencer API (NovaSeq v3.4.0 API) application.
sequencer-api.conf	/etc/httpd/clarity/ Proxy setting to communicate with the Clarity LIMS.

application.yml Properties

The application.yml file is located at opt/gls/clarity/extensions/sequencing-api/.

- All properties are configured automatically during installation, but they can also be configured by editing the application.yml file and then restarting Tomcat.
- All custom field names, both for recipe and run information, are represented by properties in the file.
- For all custom fields, the names (values in this file) can be changed. However, the property names cannot be changed, removed, or added to. For example, the Loading Workflow Type can be changed, but recipe.udfNames.sampleLoadingType cannot.

Table 171 application.yml Properties Installed

Property	Description
spring.profiles.active	Tells the application that it is running deployed in Tomcat. Default value: tomcat (Do not change)
clarity.url	Base URL that the Sequencer API service uses to contact the Clarity LIMS. The installation script (configure_ sequencer_api_application) prompts for this.
clarity.username	Username to be used when communicating with Clarity LIMS.

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Property	Description
security.signing-key	Private key that should be used when signing/validating OAuth tokens. Changing this invalidates any issued tokens.
security.token-expiry	Specifies (in hours) the expiry period for login tokens issued by the Sequencer API. Default value: 88
novaseq.sequenceStepNames	List of NovaSeq sequencing run step names for which the integration may find samples queued. • Names must be an exact match to the name of the step in Clarity LIMS (not the master step/process type). • Each line should be indented to the same point and start with a dash (-). Default value: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.3)

novaseq.flowcells	List of supported NovaSeq flow cell types.
	• Type names exactly match the values used by the NVCS run status requests.
	• To add more types, provide both type and lanes properties, indented to the same point and starting with a dash (-).
	Default value:
	`- type: "SP"`
	`lanes: 2`
	`- type: "S1"`
	`lanes: 2`
	`- type: "S2"`
	`lanes: 2`
	`- type: "S4`
	`"lanes: 4`

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Property	Description

novaseq.reagents	List of supported reagent kits to be tracked on sequencing steps.
	- lotName: The name of the lot as it appears in the run started request.
	- kitName: The name of the corresponding reagent kit in Clarity LIMS that the lot should be tracked as.
	• The name may differ in the NVCS request vs. the Clarity LIMS configuration.
	Default value:
	`- lotName: "Buffer"kitName: "Buffer Cartridge"`
	`- lotName: "Cluster"kitName: "Cluster Cartridge"`
	`- lotName: "SBS"kitName: "SBS Cartridge"`

recipe.udfNames	Configures the names of the fields used in the run recipe. See Run Recipe Contents section in *NovaSeq Run* on page 583 for more information). • Each value must be enclosed in quotes. Example: recipe.udfNames.runMode "Run Mode" • Value provided for sampleLoadingType must match the value provided in the run configuration. Example: recipe.udfNames.sampleLoadingType "Loading Workflow Type"
recipe.sampleSheet.outputName recipe.sampleSheet.notAvailableValue	Configures the name of the sample sheet file placeholder on the step where run recipe information is populated, and the property value to use when the file is not found.

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Property	Description
run.stepUdfNames	Configures the step UDF/master step field names to be used when recording run results. (See Master Step Fields section in *NovaSeq Run* on page 583 for more information.) • Each value must be enclosed in quotes. Example: run.stepUdfNames.runID "Run ID" • Value provided for sampleLoadingType must match the value provided in the run configuration. • Example: run.stepUdfNames.sampleLoadingType "Loading Workflow Type". It is expected that the run step field and the field on the input derived sample share a name.
run.metricUdfNames	Configures the result file UDFs/measurement global field names to be used when recording run results. These are the fields that capture the run data. (See *Global UDFs* section for more information.)
run.autoComplete	Determines whether the sequencing run step should be completed automatically after parsing the run metrics on a run (see also run.autoCompleteOnlyAtSuccess). Default value: true See the *Configuring Autocomplete Properties* on page 594 section for more information.
run.autoCompleteOnlyAtSuccess	Determines which of the following options the sequencing run step should be: • Autocompleted when the run status is RunCompletedSuccessfully (if autoCompleteOnlyAtSuccess is true) • Autocompleted regardless of the run status (if autoCompleteOnlyAtSuccess is false). Default value: true See the *Configuring Autocomplete Properties* on page 594 section for more information.

Reagent Categories/Label Groups

- TruSeq HT Adapters v2 (D7-D5)

Reagent Kits

- Buffer Cartridge
- Cluster Cartridge
- DPX1
- DPX2
- DPX3
- NaOH
- Resuspension Buffer (RSB)
- SBS Cartridge
- Tris HCl

Container Types

- Library Tube
- SP
- S1
- S2
- S4

This integration supports the following items:

- Library tube with barcode provided in the format [A-Za-z]{2}[0–9]{7}-[A-Za-z]{3}
  Example: AB1234567-XYZ
- SP, S1, S2, or S4 flow cell with barcode provided in one of the following formats:
  - SP and S1 flow cell: [A-Za-z0-9]{5}DRX[XY2357]
  - S2 flow cell: [A-Za-z0-9]{5}DMX[XY2357]
  - S4 flow cell: [A-Za-z0-9]{5}DSX[XY2357]

  Example: H1991DMXX

NVCS Configuration

For details on configuring NVCS for integration with Clarity LIMS, contact the Clarity LIMS Support team.

Routing Script Requirements

The requirements for the routing script functionality are as follows.

1. On the steps that use the routing script - Define Run Format (NovaSeq 6000 v3.3) and Dilute and Denature (NovaSeq 6000 v3.3) - the Next Step for all samples must be set to Remove from workflow. This value is set by a script. The value must not change in the Assign Next Steps screen.
2. In the protocol configuration screen, the following settings are required and must not be changed: In the Next Steps section, for the last step in the protocol, the method of assigning the next step must be set to Automatic.

Configuring Autocomplete Properties

As of NovaSeq Integration v3.4.0, the application.yml configuration file contains two additional properties:

- run.autoComplete
- run.autoCompleteOnlyAtSuccess

These properties determine the conditions under which the last step of the NovaSeq 6000 workflow, AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.3), is automatically completed:

- Only when the run status is "RunCompletedSuccessfully" (default behavior)

  -or-

- Regardless of the run status

By default, both properties are set to true and the step only completes if the run is successful. If the run fails or is aborted, manually complete the step in Clarity LIMS.

For the step to autocomplete regardless of the result of the sequencing run, change the autoCompleteOnlyAtSuccess property value to false.

Enabling/Disabling autoComplete Properties

1. Open the application.yml file located at /opt/gls/clarity/extensions/sequencer-api/application.yml:

```
vi /opt/gls/clarity/extensions/sequencer-api/application.yml
```

2. Edit the value of the autoCompleteOnlyAtSuccessproperty as required.
3. Save the file.

The following table shows how the combined value of the run.autoComplete and run.autoCompleteOnlyAtSuccess properties affects the autocomplete behavior of the sequencing step.

Table 172 run.autoComplete and run.autoCompleteOnlyAtSuccess Value Matrix

run.autoComplete property value	run.autoCompleteOnlyAtSuccess property value	Outcome
true (default)	true (default)	• Step autocompletes only if sequencing run is successful. • If run is not successful, step does not autocomplete and run details are recorded in Clarity LIMS.
true	false	• Step autocompletes, regardless of the run status.
false	true	• Step does not autocomplete. • Run details are recorded in Clarity LIMS.
false	false	

Rules and Constraints

- The workflow configuration contains several validation checks. To make sure that the calculations work properly, it is important that you do not disable any of this validation logic. The validation checks determine the following information:
  - Which samples, and how many, can enter each step together.
  - Which samples, and how many, can be pooled together.
- The library tube ID must be unique. There should not be multiple library tube containers in the system with the same name.
- Reagent labels (indexes) must be unique.
- Only controls are permitted as unindexed samples; all other unindexed samples and pools are not permitted.
- For sample sheet generation constraints, refer to the *Bcl2fastq2 Sample Sheet Generation* section in *Illumina Instrument Sample Sheets (NGS v5.17 & later)* on page 2542.
- Do not manually start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.3 step. This step is a fully automated and the sequencing service does not update samples correctly if they have been manually started.
- For the automated run to start successfully, the Validate Run Setup and Generate Sample Sheet button must be selected.

NovaSeq 6000 Integration v3.4.0 Validation and Troubleshooting

This section explains how to validate the installation of the Illumina NovaSeq 6000 Integration Package v3.4.0.

The validation process involves the following items:

- Running samples through the Library Prep Validation workflow.
  - The workflow contains a single-step protocol that models the library prep required to produce normalized libraries. At the end of the step, the normalized libraries advance to the workflow selected.
- Running normalized libraries through the NovaSeq 6000 v3.3 workflow validates the following items:
  - Successful routing of samples from the Run Format (NovaSeq 6000 v3.3) step to the NovaSeq Standard (NovaSeq 6000 v3.3) or NovaSeq Xp (NovaSeq 6000 v3.3) step.
  - Automated generation of v1 sample sheet for use with bcl2fastq2 v2.20 analysis software or v2 sample sheet. This file automatically uploads to the sequencing system via the Sequencer API.
  - Automatic validation of run setup information. Information is uploaded to the NovaSeq Control Software (NVCS) via the Sequencer API and is used to create the run recipe and initiate the run.
  - Automated tracking of the NovaSeq sequencing run and parsing of run statistics into Clarity LIMS, via the Sequencer API.

The validation steps assume that the Illumina NovaSeq 6000 Integration Package v3.4.0 is installed and you have imported the default Clarity LIMS configuration.

Activate Workflow, Create Project, Add and Assign Samples

The following steps set up Clarity LIMS in preparation for running samples through the Library Prep Validation and NovaSeq 6000 v3.3 workflows.

1. On the Configuration tab, under Workflows, activate both the Library Prep Validation and NovaSeq 6000 v3.3 workflows.
   - For Clarity LIMS v5.1 and later, Library Prep Validation v2.0.4 can be imported from Clarity LIMS v5.0 with IPP v2.3 installed.
2. On the Projects and Samples screen, create a project and add samples to it.
3. Assign the samples to the Library Prep Validation workflow.

Library Prep Protocol: Library Prep Validation v2.0.4

This single-step protocol models the library prep required to produce normalized libraries that are ready for the NovaSeq 6000 v3.3 workflow.

Run Library Prep Validation v2.0.4

1. In Lab View, locate the Library Prep Validation v2.0.4 protocol.
   Samples are queued for the Library Prep Validation v2.0.1 4 step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Placement screen, complete the following actions:
   a. In the Placed Samples area on the right, in the Container field, scan or enter the barcode of the destination container.
   b. Select the samples from the Samples to be Placed area on the left side, and drag them to the container wells on the right side.
   c. Select Add Labels.
5. On the Add Labels screen, the Labels to Add list is populated with labels from the TruSeq HT Adapters v2 (D7-D5) label group. The group is the only label group configured in the integration and is used by default in this step.
   a. Select labels on the left side, and drag them to the container on the right side to assign them to placed samples.
   b. Select Record Details.
6. On the Record Details screen, complete the following actions:
   a. In the Sample Details table, enter values for Normalized Molarity. Type values individually or copy and paste to enter multiple values from a spreadsheet.
   b. The Sequencing Instrument column indicates the instrument to be used for the sequencing run. Select NovaSeq 3.0.
   c. Populate the fields in this column using one of the following methods:
      - To populate fields individually for each sample, select from the drop-down list in each row.
      - To populate all fields at one time, copy and paste from a spreadsheet.

   Alternatively, select inside the table and press Ctrl + A to select all rows. At the top of the table, select Sequencing Instrument and then select NovaSeq 3.0 from the adjacent drop-down list. Select Apply.

   If working with many samples, copy and paste to insert multiple values from a spreadsheet as follows.

   a. Select Copy Samples to copy the sample information from Clarity LIMS to the Clipboard.
   b. Open a blank Excel document and paste the contents of the clipboard into the spreadsheet.
   c. In the spreadsheet, enter the Normalized Molarity and Sequencing Instrument values. Select the entire spreadsheet including the headers (Ctrl + A), and copy (Ctrl + C) the information to the Clipboard.

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d. Within Clarity LIMS, select the Paste Samples button. Paste the contents of the clipboard (Ctrl + V) into the Update Table window and then select Update to paste the contents into the Sample Details table.

7. Select Next Steps.
   Triggers the Next Steps automation, which sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step.
8. On the Assign Next Steps screen in the Samples table, the Next Step value for all samples is prepopulated with Remove from workflow.

   Do not change this value. If Next Step is not set to Remove from workflow, the routing script does not route samples correctly.

9. Select Finish Step.
   On exit from the step, the Routing Script automation is triggered. This automation assigns samples to the first step of the NovaSeq 6000 v3.3 workflow, Define Run Format (NovaSeq 6000 v3.3). This is the only step in Protocol 1: Run Format (NovaSeq 6000 v3.3).

Protocol 1: Run Format (NovaSeq 6000 v3.3)

This protocol includes a single step, Define Run Format (NovaSeq 6000 v3.3). The step allows for the assignment of per sample values for Loading Workflow Type, Normalized Molarity, Flowcell Type, and Final Loading Concentration (pM). At the end of the step, samples are routed to the NovaSeq Standard or NovaSeq Xp protocol, according to the selected Loading Workflow Type.

Step 1: Run Define Run Format (NovaSeq 6000 v3.3)

1. In Lab View, locate the Run Format (NovaSeq 6000 v3.3) protocol. The samples are queued for the Define Run Format (NovaSeq 6000 v3.3) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Record Details screen in the Sample Details table, populate the following fields (values can vary across samples):
   - Loading Workflow Type—Select NovaSeq Standard or NovaSeq Xp from the drop-down list.
   - Flowcell Type—Select SP, S1, S2, or S4.
   - Final Loading Concentration (pM)—Select from the two preset options: 225 (for PCR-free workflows), 400 (for Nano workflows), or enter a different value.
   - Normalized Molarity (nM)—These values are copied over from the previous step. If this column is not populated during library prep, enter the values here.
5. Select Next Steps, which triggers the Next Steps automation and the following actions:

- Sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step.
- Calculates the Minimum Molarity.
- Checks Normalized Molarity value. For samples with no Normalized Molarity value (in other words, empty value, not including 0), generates an error message notifying that the field cannot be empty.
- Compares each sample Normalized Molarity value with the Minimum Molarity value.

6. On the Assign Next Steps screen:
   - In the Sample Details table, the Next Step for all samples is prepopulated with Remove from workflow, regardless of the Loading Workflow Type.

     Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not routed samples correctly.

   - For samples whose Normalized Molarity value is lower than the Minimum Molarity value, the Loading Workflow Type is set to Remove from workflow. Also, a message is recorded in the Warning field for the sample.
     At this point, there are two options:
     - Return to the Record Details screen and adjust the Normalized Molarity value so that it equals or exceeds the Minimum Molarity value. The Loading Workflow Type must be set to NovaSeq Standard or NovaSeq Xp, as applicable.
     - Complete the protocol without correcting the Normalized Molarity value. In this case, the samples in question are removed from the Clarity LIMS workflow.
7. Select Finish Step. The Routing Script automation is triggered:
   - Samples whose Loading Workflow Type is set to Remove from workflow are removed from the Clarity LIMS workflow. (Occurs when the Normalized Molarity value is lower than the Minimum Molarity.)
   - Samples whose Loading Workflow Type is set to NovaSeq Standard are routed to the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.3) step. This is the first step in the NovaSeq Standard protocol.
   - Samples whose Loading Workflow Type is set to NovaSeq Xp are routed to the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.3) step. This is the first step in the NovaSeq Xp protocol.

Protocol 2: NovaSeq Standard (NovaSeq 6000 v3.3)

In this protocol, samples are pooled and added to the library tube in preparation for the NovaSeq run. The protocol contains two steps:

1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.3)
2. Dilute and Denature (NovaSeq 6000 v3.3)

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Step 1: Run Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.3)

1. In Lab View, locate the NovaSeq XP (NovaSeq 6000 v3.3) protocol. Samples for the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.3) step are queued.
2. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Pooling screen:
   a. Create a pool by dragging samples into the Pool Creator.

      Only create one pool.

   b. Enter a name for the pool or accept the default name (Pool #1).
   c. Select Record Details.
      After exiting from the Pooling screen, the Validate Inputs Flowcell Type and Single Pool is triggered. The automation verifies the following items:
      - All samples in the pool have been assigned the same Flowcell Type.
      - Only one pool has been created.
5. On the Record Details screen in the Step Details area, complete the two required fields and one optional field, as needed:
   - Number of Flowcells to Sequence—Used in volume calculations, to make sure that the volumes are sufficient for the number of times the pool is sequenced.
   - Minimum Per Sample Volume (µl)—Used to calculate how much of each sample is included in the pool. The field is prepopulated with the configured default value 5 µl, but can be edited.
   - % PhiX (2.5 nM) Spike-In—Used to calculate the volume of PhiX v3 control included in the pool for the given percentage of spike-in. The field is optional and can be edited.

     Assuming the default Minimum Per Sample Volume (µl) value of 5, for a given batch:
     - If the smallest Per Sample Volume (µl) value is < 5, Clarity LIMS automatically assigns a value of 5 to the Adjusted Per Sample Volume (µl) field.
     - Clarity LIMS changes the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.
6. In the Sample Details table, select the pool icon to view details on the pool composition.
7. Select Calculate Volumes to trigger the Calculate Volumes automation. This automation performs calculations based on the selected Flowcell Type.
   - Calculates Bulk Pool Volume (µl) value.
   - Sets the value of the Bulk Pool Volume (µl) field.

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- Calculates the PhiX Volume (µl) value if % PhiX (2.5 nM) Spike-In has value.
- Calculates the Per Sample Volume (µl) value to be added to the pool.
- Calculates the Total Sample Volume (µl) value.
- If the Total Sample Volume is less than the Bulk Pool Volume, selects the RSB Volume (µl) value.
- Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
- Populates the Volume of Pool to Denature (µl), NaOH Volume (µl) and Tris-HCl Volume (µl) columns of the Sample Details table. The script sets the values, and is not editable.
- The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains volume information about the pool and the individual samples it contains. Select on the file to download it and then open it in Excel.

8. Select Next Steps to trigger the Set Next Step automation. This automation sets the next step for samples to ADVANCE and advances them to the next step in the protocol, Dilute, and Denature (NovaSeq 6000 v3.3).
   On the Assign Next Steps screen, the next step for samples is set to the next step in the workflow: Dilute and Denature (NovaSeq 6000 v3.3).
9. Select Finish Step.
   At the end of this step, the pool of samples automatically advances to the Dilute and Denature (NovaSeq 6000 v3.3) step.

Step 2: Run Dilute and Denature (NovaSeq 6000 v3.3)

1. In Lab View, locate the NovaSeq Standard (NovaSeq 6000 v3.3) protocol. The pool of samples queued for the Dilute and Denature (NovaSeq 6000 v3.3) step are listed.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work. The Validate Single Input automation is triggered. This automation verifies that only one container is input for the step.
4. On the Placement screen:
   a. Drag the pool into the library tube in the Placed Samples area.
   b. Scan or type the barcode of the library tube into the Library Tube field.
   c. Select Record Details.
   After exiting the Placement screen, the Validate Library Tube Barcode automation makes sure that the library tube barcode conforms to the barcode mask [A-Za-z]{2}[0-9]{7}-[A-Za-z]{3}. If it does not, an error message displays. The automation also copies the Flowcell Type and Loading Workflow Type values from step inputs to outputs.
5. On the Record Details screen, the Reagent Lot Tracking section lets you tracks the NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. Add and activate lots for these reagents.

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To add and activate reagent lots:

a. In a new browser tab, open an additional instance of Clarity LIMS.
b. Navigate to the Consumables configuration screen.
c. Select the NaOH reagent kit and select New Lot.
d. Enter the lot details and select Save.
e. Set the Status of Reagent Lot toggle switch to Active.
f. Repeat this process to add and activate Resuspension Buffer and Tris-HCl reagent lots.
g. Return to your original working browser tab and refresh the page.

6. In the Reagent Lot Tracking section, select from the active lots displayed in each drop-down list.
7. The fields displayed on the Record Details screen are used to set up the run and generate the sample sheet. Some of these fields are autopopulated and some must be completed manually.

Table 173 Fields Displayed on Record Details Screen of Denature and Dilute (NovaSeq 6000 v3.3)

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.
BaseSpace Sequence Hub Configuration	Select from preset options: Not Used, Run Monitoring Only, or Run Monitoring and Storage.
Workflow	Automatically populated with the preset GenerateFASTQ.
Workflow Type	Select from preset options: No Index, Single Index, Dual Index, or Custom.
Index Read 1	Select from preset options: 0, 6, or 8, or type a value between 0 and 20.
Index Read 2	Select from preset options: 0, 6, or 8, or type a value between 0 and 20.
Paired End	Select from preset options: True or False.
Read 1 Cycles	Select from preset options: 251, 151, 101, or 51, or type a value between 1 and 251. *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Read 2 Cycles	Select from preset options: 251, 151, 101, or 51, or type a value between 1 and 251. *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Use Custom Read 1 Primer	Select if applicable.

Field	Value
Use Custom Read 2 Primer	Select if applicable.
Use Custom Index Read 1 Primer	Select if applicable.
Reverse Complement Workflow	Select Yes (by default) for v1.5 reagents in NovaSeq Control Software v1.7 and onwards. Select No for v1 reagents.
Use Custom Recipe	Select if applicable.
Custom Recipe Path	If the Use Custom Recipe option is selected, enter the path to the custom recipe file to be used.
UMI - Read 1 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 1. Leave blank if not applicable.
UMI - Read 2 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 2. Leave blank if not applicable.
UMI - Read 1 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.
UMI - Read 2 Start From Cycle	Enter the cycle number that Read 2 starts from. Leave blank if not applicable.
Output Folder	Enter network path for sequencing run folder. For example: \\networkshare\run_data
Samplesheet Format	Select from preset options: V1 or V2
Analysis Software Version	Enter the software version for BCL Convert Analysis for v2 sample sheet. Leave blank if not applicable.
Override Cycles	Enter the Override Cycles value for v2 sample sheet. Leave blank if not applicable.

8. On the Record Details screen, select Validate Run Setup and Generate Sample Sheet. This triggers the automation script, which performs the following actions:
   - Validates the parameters entered on the Record Details screen.
   - Generates the sample sheet and attaches it to the placeholder in the Files area of the Record Details screen.

The v2 sample sheet generated does not include Cloud and Analysis sections. Contact Illumina Support for information on generating v2 sample sheets with those sections.

   - Select Next Steps.

9. On the Assign Next Steps screen, the Next Step for samples is prepopulated with Remove from workflow. The Routing Script automation expects this value, and requires the value to advance samples to the next step.

   Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.

10. Select Finish Step.
    After exiting the step, the following actions occur:
    - The Routing Script automation is triggered and samples are routed to AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.3).
    - In Lab View, the pool of samples is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.3) step.

    At this point in the workflow, the user interaction ends. Proceed to AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.3).

    Do not add samples to the Ice Bucket or start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.3) step. The integration adds samples automatically.

Protocol 3: NovaSeq Xp (NovaSeq 6000 v3.3)

In this protocol, samples are pooled and added to lanes on the NovaSeq flow cell. The flow cell type is determined by the option selected in the Define Run Format (NovaSeq 6000 v3.3) step.

The protocol contains three steps:

1. Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.3)
2. Dilute, Denature, and ExAmp (NovaSeq 6000 v3.3)
3. Load to Flowcell (NovaSeq 6000 v3.3)

Step 1: Run Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.3)

1. In Lab View, locate the NovaSeq Xp (NovaSeq 6000 v3.3) protocol. The samples are queued for the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.3) step.
2. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Pooling screen:
   a. Create a pool by dragging samples into the Pool Creator.

      Create only one pool.

   b. Enter a name for the pool or accept the default name (Pool #1).
   c. Select Record Details.

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5. On exiting the Pooling screen, the Validate Inputs Flowcell Type and Single Pool automation is triggered. The automation verifies the following items:
   - All samples in the pool are assigned the same Flowcell Type.
   - Only one pool was created.
6. On the Record Details screen in the Step Details area, complete the two required fields and one optional field, as needed:
   - Number of Lanes to Sequence—Used in volume calculations to make sure that volumes are sufficient for the number of times the pool is sequenced.
   - Minimum Per Sample Volume (µl)—Used to calculate how much of each sample is included in the pool. The field is prepopulated with the configured default value 5 µl, but can be edited.
   - % PhiX (0.25nM) Spike-In—Used to calculate the volume of PhiX v3 control to be included in the pool for the given percentage of spike-in. The field is optional and can be edited.

     Assuming the default Minimum Per Sample Volume (µl) value of 5, for a given batch:
     - If the smallest Per Sample Volume (µl) value is < 5, Clarity LIMS automatically assigns a value of 5 to the samples Adjusted Per Sample Volume (µl) field.
     - Clarity LIMS then adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.
7. In the Sample Details table, select the pool icon to view details on the pool composition.
8. Select Calculate Volumes. This triggers the Calculate Volumes automation. This automation performs volume calculations based on the selected Flowcell Type, as follows.
   - Calculates Bulk Pool Volume (µl) value.
   - Calculates the Per Sample Volume (µl) value to be added to the pool.
   - Calculates PhiX Volume (ul) value if % PhiX (0.25nM) Spike-In has value.
   - Calculates the Total Sample Volume (µl) value.
   - If the Total Sample Volume is less than the Bulk Pool Volume, calculate the RSB Volume (µl) value.
   - Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
   - Generates the Calculation File (CSV) and attaches it to the step. This file contains volume information about the samples and RSB buffer to add to the pool. Select the file to download it and then open it in Excel.
9. Select Next Steps to trigger the Set Next Step automation. This automation performs the following actions:
   - Copies the Flowcell Type values from the step inputs to the step outputs.

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- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol Dilute, Denature, and ExAmp (NovaSeq 6000 v3.3).

On the Assign Next Steps screen, the next step for samples is set to the next step in the workflow Dilute, Denature, and ExAmp (NovaSeq 6000 v3.3).

10. Select Finish Step.
    At the end of this step, the pool of samples automatically advances to the Dilute, Denature, and ExAmp (NovaSeq 6000 v3.3) step.

Step 2: Run Dilute, Denature and ExAmp (NovaSeq 6000 v3.3)

1. In Lab View, locate the NovaSeq Xp (NovaSeq 6000 v3.3) protocol. The pool of samples queued for the Dilute, Denature, and ExAmp (NovaSeq 6000 v3.3) step displays.
2. Add the pool to the Ice Bucket and select View Ice Bucket.
3. [Optional] On the Ice Bucket screen, set the number of derivatives to create (placed into the flow cell lanes) and select Begin Work.
4. On entry to the Record Details screen, the Calculate Volumes automation is triggered.
   This automation sets the following values based on the selected Flowcell Type:
   - BP Aliquot Volume (µl)
   - NaOH Volume (µl)
   - Tris-HCl Volume (µl)
   - DPX1 Volume (µl)
   - DPX2 Volume (µl)
   - DPX3 Volume (µl)
   - Mastermix per Lane (µl)
   - The automation also populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
   - The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains information about the DPX Mastermix volume and the volume of Mastermix, NaOH, and Tris-HCl to add per working pool (see the next step).
5. On the Record Details screen, the Reagent Lot Tracking section tracks the DPX1, DPX2, DPX3, NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. These reagents must be added.
   To add and activate reagent lots:
   a. In a new browser tab, open an additional instance of Clarity LIMS.
   b. Select one of the reagent kits previously listed and select New Lot.
   c. Enter the lot details and select Save.

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d. Set the Status of Reagent Lot toggle switch to Active.
e. Repeat this process to add and activate the remaining reagent lots.
f. Return to your original working browser tab and refresh the page.

6. On the Record Details screen:
   - In the Reagent Lot Tracking section, select from the active lots displayed in each drop-down list.
   - In the Step Details area, the DPX1, DPX2, and DPX3 reagent volume values are prepopulated. The script sets the values and they are not editable.
   - In the Sample Details table, select the pool icon to view details on the working pool composition.
     - The BP Aliquot, Mastermix per lane, NaOH, and Tris-HCl volume values for each working pool are populated. The script sets the values and they are not editable.
     - The Flowcell Type and Loading Workflow Type columns are populated.
     - The working pool number is appended to the bulk pool name, to identify which working pools are derived from the same bulk pool.
7. In the Files area, select the Calculation File (CSV) to open it and view details on the DPX Mastermix volume and the volume of Mastermix, NaOH, and Tris-HCl to add per working pool.
8. Select Next Steps. On the Assign Next Steps screen, the next step is already set to Load to Flowcell (NovaSeq 6000 v3.3).
9. Select Finish Step.

Step 3: Run Load to Flowcell (NovaSeq 6000 v3.3)

1. On the Ice Bucket screen:
   - In the Container Options panel, select the appropriate flow cell type from the Destination Container drop-down list.
   - Select Begin Work.
2. The Validate Inputs and Selected Container automation checks the following:
   - The Flowcell Type field is set to a valid value (SP, S1, S2, or S4) and that each input has the same value for the field.
   - The container type selected matches the value in the Flowcell Type field.
   - The number of outputs matches the number of lanes on the selected flow cell type. If validation fails, an error message reports the number of working pools does not match the number of lanes available on the flow cell.
3. On the Placement screen:
   - Drag one or more pools from the left of the screen over into the Placed Samples area on the right.

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- Scan or type the barcode of the flow cell into the Flowcell field.
- Select Record Details.

After exiting the Placement screen, the Validate Flowcell Barcode automation makes sure that the container barcode conforms to the barcode mask for the selected Flowcell Type (as listed in the following table). This automation also copies the Flowcell Type and Loading Workflow Type field values from step inputs to outputs.

Flowcell Type	Barcode Mask
SP	[A-Za-z0-9]{5}DRX[XY2357]
S1	[A-Za-z0-9]{5}DRX[XY2357]
S2	[A-Za-z0-9]{5}DMX[XY2357]
S4	[A-Za-z0-9]{5}DSX[XY2357]

4. The fields displayed on the Record Details screen are used to set up the run and generate the sample sheet.
Some of these fields are autopopulated and some must be entered manually (see the following table for details).

Table 174 Fields Displayed on Record Details Screen of Load to Flowcell (NovaSeq 6000 v3.3) Step

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. No spaces.
BaseSpace Sequence Hub Configuration	Select from preset options: Not Used, Run Monitoring Only, or Run Monitoring and Storage.
Workflow	Automatically populated with preset - GenerateFASTQ.
Workflow Type	Select from preset options: No Index, Single Index, Dual Index, or Custom.
Index Read 1	Select from preset options: 0, 6, or 8 - or type a value between 0 and 20.
Index Read 2	Select from preset options: 0, 6, or 8 - or type a value between 0 and 20.
Paired End	Select from preset options: True or False.
Read 1 Cycles	Select from preset options: 251, 151, 101, or 51 - or type a value between 1 and 251. *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.

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Field	Value
Read 2 Cycles	Select from preset options: 251, 151, 101, or 51 - or type a value between 1 and 251. *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Use Custom Read 1 Primer	Select if applicable.
Use Custom Read 2 Primer	Select if applicable.
Use Custom Index Read 1 Primer	Select if applicable.
Reverse Complement Workflow	Select Yes (by default) for v1.5 reagents in NovaSeq Control Software v1.7 and onwards. Select No for v1 reagents.
Use Custom Recipe	Select if applicable.
Custom Recipe Path	If you selected the Use Custom Recipe option, enter the path to the custom recipe file to be used.
UMI - Read 1 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 1. Leave blank if not applicable.
UMI - Read 2 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 2. Leave blank if not applicable.
UMI - Read 1 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.
UMI - Read 2 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.
Output Folder	Enter network path for sequencing run folder. For example: \\networkshare\run_data
Library Tube Barcode	Scan the library tube barcode.
Samplesheet Format	Select from preset options: V1 or V2
Analysis Software Version	Enter the software version for BCL Convert Analysis for v2 sample sheet. Leave blank if not applicable.
Override Cycles	Enter the Override Cycles value for v2 sample sheet. Leave blank if not applicable.

5. Select Validate Run Setup and Generate Sample Sheet, then select Next Steps to trigger the automation script, which:
   - Validates the parameters entered on the Record Details screen.

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- Generates the sample sheet and attaches it to the placeholder in the Files area on the Record Details screen.

The v2 sample sheet generated does not include Cloud and Analysis sections. Contact Illumina Support for information on generating v2 sample sheets with these sections.

6. Select Next Steps.
   On the Assign Next Steps screen, the Next Step field for samples is prepopulated with Mark protocol as complete.
7. Select Finish Step.
   At this point in the workflow, the user interaction ends. The flow cell is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.3) step.

Proceed to AUTOMATED - .NovaSeq Run (NovaSeq 6000 v3.3).

Do not add samples to the Ice Bucket or start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.3) step. The integration adds samples automatically.

Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.3)

This protocol contains a single fully automated step - AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.3).

The integration starts the step automatically and data from the run is parsed back into Clarity LIMS. User interaction is not required, but the various stages can be reviewed in Clarity LIMS (see Review Run Data).

Automations

The NovaSeq Run (NovaSeq 6000 v3.3) step contains two automations:

- Set Next Steps—This automation is disabled (Set to Not Used). By default, the Sequencer API is used to set the next step for output samples.
  The Illumina Preset Protocols (IPP) include a Set Next Steps automation configured on the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.3) step. You can use this automation to override the default next step behavior. See *NovaSeq 6000 Integration v3.4.0 Configuration and User Interaction* on page 536for details.
- Update Lane Number—This automation is triggered automatically upon entry to the Record Details screen.
  Do not disable or modify this automation to make sure that the Lane Number displays properly.

Review Run Data

Read summary metrics are recorded for the library pool. After the run is complete, open the step and review these metrics on the Next Steps screen in the Step Data section and the Sample Details table.

Step Data Section

The following values populate in the step UDFs/master step fields:

- Run ID
- Run Status
- Output Folder
- Current Cycle
- Current Read
- Loading Workflow Type
- Flow Cell ID
- Flow Cell Side
- Flow Cell Mode
- Flow Cell Part Number*
- Flow Cell Lot Number
- Flow Cell Expiration Date
- Instrument ID
- Instrument Type
- Instrument Control Software Version
- Firmware Version
- RTA Version
- Sequencing Log

Sample Details Table

Summary metrics populate the UDFs/global fields listed. Values are aggregated across all lanes. Some values (for example Yield PF (Gb) R1) are summed, while others are averaged.

- Yield PF (Gb) R1
- Yield PF (Gb) R2*
- Reads PF (M) R1*
- Reads PF (M) R2*
- Cluster Density (K/mm^2) R1
- Cluster Density (K/mm^2) R2
- %PF R1
- %PF R2

- % Bases >=Q30 R1
- % Bases >=Q30 R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- % Aligned R1
- % Aligned R2
- % Error Rate R1
- % Error Rate R2

How the Integration Works

The following steps summarize how the Sequencer API integration works. For details on sample sheet generation and the sequence of events involved in the NovaSeq run, refer to *NovaSeq 6000 Integration v3.4.0 Configuration and User Interaction* on page 536.

1. When setting up a run on the NovaSeq instrument, NVCS sends a request for the run recipe.
   a. The Sequencer API validates that samples and containers are correctly queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.3 step.
   b. Clarity LIMS sends a JSON response to NVCS. The response includes the run recipe information and a link to download the sample sheet (used with analysis software such as bcl2fastq2).
2. When the run starts, NVCS sends a RunStarted run status request. The Sequencer API then does the following:
   a. Validates reagent kit information in the RunStarted request:
      - Verifies that the reagent kit exists. If the reagent kit does not exist, the Sequencer API creates and enables it on the master step.
      - Verifies that the reagent kit is activated. If the reagent kit is not active, the Sequencer API activates it.
   b. Initiates the step for the queued samples. The step produces one output file placeholder per lane of the flow cell in use, based on the flow cell type.
   c. Records all relevant information from the RunStarted request on the step (such as reagent lots and step fields).
3. At the end of the run, NVCS sends one of the following run status requests:
   - RunCompletedSuccessfully

- RunEndedByUSer
- RunErroredOut

The Sequencer API updates the step with any new information, which includes only the status and cycle/read information.

4. After primary analysis completes, NVCS sends a request containing the parsed run metrics. NVCS sends a request only when the run completed successfully, but the API does not make this assumption and accepts the request regardless of status. The Sequencer API then does the following:
   a. Records the metrics into the fields on file placeholder outputs in the LIMS.
   b. Completes the step in Clarity LIMS if the status is RunCompletedSuccessfully. For all other status options, the step remains in progress. This action is the default autocomplete step behavior. Refer to *NovaSeq 6000 Integration v3.4.0 Configuration and User Interaction* on page 536 for details.

   > *NovaSeq 6000 Integration v3.4.0 Configuration and User Interaction* on page 536 provides detailed information on how the following components work with the Sequencer API integration:
   >
   > - Sample sheet generation and contents
   > - NovaSeq run
   > - User authentication and login
   > - Run recipe contents

Troubleshooting

If an automation trigger does not appear to run its corresponding scripts, see the following sections in the Clarity LIMS API documentation:

- Troubleshooting Automated Informatics
- Troubleshooting Automation in the API

If an error occurs that does not provide direction on how to proceed, complete the following steps:

1. Confirm the version of the installed Illumina NovaSeq Integration Package by running the following command on the Clarity LIMS server command line:

```
rpm -qa | grep -i novaseq
```

2. If the error is related to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.3) step, review the log file information. For details, refer to *Logging* on page 614.

If the automated run step starts, but does not complete:

1. Use one of the following methods to open the in progress step in Clarity LIMS:
   - Log in to the default user account.
   - In Lab View, find the step in the Recent Activities pane.

- Search for the step in Clarity LIMS, using either the Library Tube, or Flowcell barcode as the search term. The steps depend on whether the search is a NovaSeq Standard or NovaSeq Xp run.

2. On the Record Details screen, the Sequencing Log multiline text field contains logging information. If unable to reach the Record Details screen, or if the Sequencing Log field does not contain enough information to resolve the issue, review the sequencer-api.log file. For details, refer to *Logging* on page 614.
3. Contact the Clarity LIMS support team. Provide the relevant information from the troubleshooting steps already performed.

Logging

In addition to updating the Sequencing Log multiline text field on the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.3) step, the Sequencer API writes a detailed log file to /opt/gls/clarity/tomcat/current/logs/sequencer-api.log.

Log messages include the Library Tube ID and Flowcell ID whenever the messages are related to a sequencing run-related request. In the LIMS workflows, these IDs are recorded as container names.

Some log messages are not directly related to a sequencing run request, such as downloading the sample sheet. In this case, the file LIMS ID and file name are included in log messages. The sample sheet downloaded by the sequencing system has a name that includes the sequencing container ID (Library Tube for Standard or Flowcell for Xp). The relevant container name search finds most the log messages for this request. However, if all messages for downloading a file are found, then the LIMS ID of the file must be known. The LIMS ID is found in the Clarity LIMS API and, for sample sheets used in a run, in the recipe response (which can also be found in the log file).

Example lines from the log file:

```
2021-06-19 21:32:19.705 INFO --- [] SequencerAPIApplication : Started
SequencerAPIApplication in 7.026 seconds (JVM running for 15.964)
...
2021-06-19 21:34:44.564 INFO --- [Library Tube Id=NV1234567-LIB, Flow Cell
Id=RM123DSXX] LimsApiLookupUtil : Searching for containers with name 'NV1234567-
LIB' or 'RM123DSXX'
....
2021-06-19 21:35:27.158 INFO --- [] FileService : Successfully retrieved file
'NV1234567-LIB.csv' with LIMS ID 40-51 through the API.
```

NovaSeq 6000 Integration v3.4.0 Manual Upgrade

NovaSeq Integration Package v3.4.0 supports future ICA services along with NovaSeq Control Software v1.8 (with the ICA integration feature). As a result, there are new custom fields and a new sample sheet template file. There are also modifications to the existing automations and step configurations.

If you do not want to upgrade the existing NovaSeq Sequencing v3.2 workflow to the latest version through Illumina Preset Protocols (IPP), you can update the workflow configuration manually by completing the following steps.

Create a Custom Field

1. In Clarity LIMS, under Configuration, select the Custom Fields tab.
2. Under the Global Fields tab, select Submitted Sample.
3. Create a custom field named Illumina Universal Sample Identifier.
4. For Field Type, select Text.
5. For Field Options, select the following:
    - Required Field—No
    - Read Only—No
6. Select Save.

Modify the Existing NovaSeq v3.2 Workflow

Create custom fields for master steps in the workflow as follows.

1. From Configuration, select the Custom Fields tab.
2. Under the Master Step Fields tab, select the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.2) master step.
3. Create a custom field named Run Completion Date.
4. For Field Type, select Text.
5. For Field Options, select the following:
    - Required Field—No
    - Read Only—Yes
6. Under the Master Step Fields tab, select the Dilute and Denature (NovaSeq 6000 v3.2) master step.
7. Create custom fields using the following table.

Table 175 Custom Fields for Dilute and Denature (NovaSeq 6000 v3.2) Master Step

Name	Field Type	Field Options	Additional Options
Analysis Software Version	Text	• Required—No • Read Only—No	Not applicable
Override Cycles	Text	• Required—No • Read Only—No	Not applicable

Name	Field Type	Field Options	Additional Options
Samplesheet Format	Text Dropdown	• Required—Yes • Read Only—No • Custom Entries—No	• Dropdown Items—V1, V2 • For V1, select Yes under Set as Default
Validation Script	Multiline Text	• Required—Yes • Read Only—Yes	Default value provided in the drop-down section following the table.

Validation Script

```
step.::Run Mode:: = input.::Flowcell Type::; if (!step.::Experiment
Name::.matches(::[a-zA-Z0-9-_]+::)) { fail(::Experiment Name contains prohibited
characters. Allowed characters are: a-z, A-Z, 0-9, -, and _::) }; if
(step.::Workflow Type:: == ::No Index::) { if (step.::Index Read 1:: != 0 ||
step.::Index Read 2:: != 0) { fail(::Index Read 1 and Index Read 2 must be 0 if
No Index is selected.::) } } else if (step.::Workflow Type:: == ::Single
Index::) { if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) { fail
(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if Single
Index is selected.::) } } else { if (step.::Index Read 1:: == 0 || step.::Index
Read 2:: == 0) { fail(::Index Read 1 and Index Read 2 must be greater than 0 if
Dual Index is selected.::) } }; if (step.::Paired End::.toBoolean()) { if
(step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) { fail(::Read 1
Cycles and Read 2 Cycles must be greater than 0 if Paired End is True.::) } }
else { if (step.::Read 2 Cycles:: != 0) { fail(::Read 2 Cycles must be 0 if
Paired End is False.::) }; if (step.hasValue(::UMI - Read 2 Length::) ||
step.hasValue(::UMI - Read 2 Start From Cycle::)) { fail(::UMI - Read 2 Length
and UMI - Read 2 Start From Cycle cannot be defined if Paired End is False.::) }
}; if (input.::Flowcell Type:: != ::SP:: && step.::Read 1 Cycles:: > 151) { fail
(::Read 1 Cycles must not be larger than 151 if it is not SPrime Flowcell::) };
if (input.::Flowcell Type:: != ::SP:: && step.::Read 2 Cycles:: > 151) { fail
(::Read 2 Cycles must not be larger than 151 if it is not SPrime Flowcell::) };
if (step.hasValue(::UMI - Read 1 Length::) && !step.hasValue(::UMI - Read 1
Start From Cycle::)) { fail(::UMI - Read 1 Start From Cycle must be greater than
0 if UMI - Read 1 Length is greater than 0.::) }; if (!step.hasValue(::UMI -
Read 1 Length::) && step.hasValue(::UMI - Read 1 Start From Cycle::)) { fail
(::UMI - Read 1 Length must be greater than 0 if UMI - Read 1 Start From Cycle
is greater than 0.::) }; if (step.hasValue(::UMI - Read 2 Length::) &&
!step.hasValue(::UMI - Read 2 Start From Cycle::)) { fail(::UMI - Read 2 Start
From Cycle must be greater than 0 if UMI - Read 2 Length is greater than 0.::)
}; if (!step.hasValue(::UMI - Read 2 Length::) && step.hasValue(::UMI - Read 2
Start From Cycle::)) { fail(::UMI - Read 2 Length must be greater than 0 if UMI
```


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```
- Read 2 Start From Cycle is greater than 0.::) }; if (!step.hasValue(::UMI -
Read 1 Length::) && step.hasValue(::UMI - Read 2 Length::)) { fail(::UMI - Read
1 Length must be greater than 0 if UMI - Read 2 Length is greater than 0.::) };
if (!step.hasValue(::UMI - Read 1 Start From Cycle::) && step.hasValue(::UMI -
Read 2 Start From Cycle::)) { fail(::UMI - Read 1 Start From Cycle must be
greater than 0 if UMI - Read 2 Start From Cycle is greater than 0.::) }; if
(step.hasValue(::Analysis Software Version::) && !step.::Analysis Software
Version::.matches(::^(\\d+)(\\.\\d+)*$::)) { fail(::Analysis Software Version
contains prohibited characters. Allowed characters are 0-9 and period. It shall
start and end with numbers and separated by single period e.g. 3.8.4::) }; if
(step.hasValue(::Override Cycles::) && !step.::Override Cycles::.matches(::
[YNIU0-9;]+::)) { fail(::Override Cycles contains prohibited characters. Allowed
characters are: Y, N, I, U, 0-9 and ;. Example: N1Y150;I8;I7N1;Y141U10.::) };
step.::Settings Header:: = step.hasValue(::UMI - Read 1 Length::) ||
step.hasValue(::UMI - Read 2 Length::) ? ::[Settings]:: : ::::; nextStep =
::REMOVE::
```

8. Select Save.
9. Under the Master Step Fields tab, select the Load to Flowcell (NovaSeq 6000 v3.2) master step.
10. Create custom fields using the following table.

Table 176 Custom Fields for Load to Flowcell (NovaSeq 6000 v3.2) Master Step

Name	Field Type	Field Options	Additional Options
Analysis Software Version	Text	• Required—No • Read Only—No	Not applicable
Override Cycles	Text	• Required—No • Read Only—No	Not applicable
Samplesheet Format	Text Dropdown	• Required—Yes • Read Only—No • Custom Entries—No	• Dropdown Items—V1, V2 • For V1, select Yes under Set as Default
Validation Script	Multiline Text	• Required—Yes • Read Only—Yes	Default value provided in the drop-down section following the table.

Validation Script

```
step.::Run Mode:: = input.::Flowcell Type::; if (!step.::Experiment
Name::.matches(::[a-zA-Z0-9-_]+::)) { fail(::Experiment Name contains prohibited
characters. Allowed characters are: a-z, A-Z, 0-9, -, and _::) }; if
(step.::Workflow Type:: == ::No Index::) { if (step.::Index Read 1:: != 0 ||
```


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```
step.::Index Read 2:: != 0) { fail(::Index Read 1 and Index Read 2 must be 0 if
No Index is selected.::) } } else if (step.::Workflow Type:: == ::Single
Index::) { if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) { fail
(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if Single
Index is selected.::) } } else { if (step.::Index Read 1:: == 0 || step.::Index
Read 2:: == 0) { fail(::Index Read 1 and Index Read 2 must be greater than 0 if
Dual Index is selected.::) } }; if (step.::Paired End::.toBoolean()) { if
(step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) { fail(::Read 1
Cycles and Read 2 Cycles must be greater than 0 if Paired End is True.::) } }
else { if (step.::Read 2 Cycles:: != 0) { fail(::Read 2 Cycles must be 0 if
Paired End is False.::) }; if (step.hasValue(::UMI - Read 2 Length::) ||
step.hasValue(::UMI - Read 2 Start From Cycle::)) { fail(::UMI - Read 2 Length
and UMI - Read 2 Start From Cycle cannot be defined if Paired End is False.::) }
}; if (input.::Flowcell Type:: != ::SP:: && step.::Read 1 Cycles:: > 151) { fail
(::Read 1 Cycles must not be larger than 151 if it is not SPrime Flowcell::) };
if (input.::Flowcell Type:: != ::SP:: && step.::Read 2 Cycles:: > 151) { fail
(::Read 2 Cycles must not be larger than 151 if it is not SPrime Flowcell::) };
if (step.hasValue(::UMI - Read 1 Length::) && !step.hasValue(::UMI - Read 1
Start From Cycle::)) { fail(::UMI - Read 1 Start From Cycle must be greater than
0 if UMI - Read 1 Length is greater than 0.::) }; if (!step.hasValue(::UMI -
Read 1 Length::) && step.hasValue(::UMI - Read 1 Start From Cycle::)) { fail
(::UMI - Read 1 Length must be greater than 0 if UMI - Read 1 Start From Cycle
is greater than 0.::) }; if (step.hasValue(::UMI - Read 2 Length::) &&
!step.hasValue(::UMI - Read 2 Start From Cycle::)) { fail(::UMI - Read 2 Start
From Cycle must be greater than 0 if UMI - Read 2 Length is greater than 0.::)
}; if (!step.hasValue(::UMI - Read 2 Length::) && step.hasValue(::UMI - Read 2
Start From Cycle::)) { fail(::UMI - Read 2 Length must be greater than 0 if UMI
- Read 2 Start From Cycle is greater than 0.::) }; if (!step.hasValue(::UMI -
Read 1 Length::) && step.hasValue(::UMI - Read 2 Length::)) { fail(::UMI - Read
1 Length must be greater than 0 if UMI - Read 2 Length is greater than 0.::) };
if (!step.hasValue(::UMI - Read 1 Start From Cycle::) && step.hasValue(::UMI -
Read 2 Start From Cycle::)) { fail(::UMI - Read 1 Start From Cycle must be
greater than 0 if UMI - Read 2 Start From Cycle is greater than 0.::) }; if
(step.hasValue(::Analysis Software Version::) && !step.::Analysis Software
Version::.matches(::^(\\d+)(\\.\\d+)*$::)) { fail(::Analysis Software Version
contains prohibited characters. Allowed characters are 0-9 and period. It shall
start and end with numbers and separated by single period e.g. 3.8.4::) }; if
(step.hasValue(::Override Cycles::) && !step.::Override Cycles::.matches(::
[YNIU0-9;]+::)) { fail(::Override Cycles contains prohibited characters. Allowed
characters are: Y, N, I, U, 0-9 and ;. Example: N1Y150;I8;I7N1;Y141U10.::) };
step.::Settings Header:: = step.hasValue(::UMI - Read 1 Length::) ||
```


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```
step.hasValue(::UMI - Read 2 Length::) ? ::[Settings]:: : ::::; nextStep =
::ADVANCE::
```

11. Select Save.

Update Step File Placeholder

1. From Configuration, select the Lab Work tab.
2. In the Master Steps list, select the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.2) master step.
3. Select the Record Details milestone.
4. In Step File Placeholders, create a Log File.
5. In the Master Steps list, select the Define Run Format (NovaSeq 6000 v3.2) step.
6. Select the Record Details milestone.
7. In Step File Placeholders, change the first placeholder name to Log File.
8. In the Master Steps list, select the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.2) master step.
9. Select the Record Details milestone.
10. In Step File Placeholders, change the second placeholder name to Log File.
11. In the Master Steps list, select the Make Bulk Pool for NovaSeq XP (NovaSeq 6000 v3.2) master step.
12. Select the Record Details milestone.
13. In Step File Placeholders, change the second placeholder name to Log File.

Modify Existing Automations

Update the Lane Number automation on the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.2) step as follows.

1. From Configuration, select the Automation tab.
2. Under the Step Automation tab, select the Update Lane Number automation enabled on the Under the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.2) step.
3. Change `compoundOutputFileLuid3` to `compoundOutputFileLuid0` in the existing command line as follows.
   Changes to the existing command line are in red.

```
bash -l -c "sleep 10 && /opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2}
-u {username} -p {password} \
script:evaluateDynamicExpression \
```

```
-exp 'if (input.::Loading Workflow Type:: == ::NovaSeq Standard::) {
output.::name:: = ::Lane :: + step.::Lane Counter::; output.::name:: =
output.::name::.split(::\\.::)[0] + :::1::; step.::Lane Counter::++; }' \
-log {compoundOutputFileLuid0} \
-t true \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-exp 'step.::Lane Counter:: = 1' \
-log {compoundOutputFileLuid0} \
-t true"
```

4. Select Save.

Update the Validate Run Setup and Generate Sample Sheet automation on the Dilute and Denature (NovaSeq 6000 v3.2) step as follows.

1. From Configuration, select the Automation tab.
2. Under the Step Automation tab, select the Validate Run Setup and Generate Sample Sheet automation enabled on the Dilute and Denature (NovaSeq 6000 v3.2) step.
3. Update the existing command line as follows.
   Changes to the existing command line are in red.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-exp '{udf:Validation Script}' \
-log {compoundOutputFileLuid1} \
-t true \
&& if [ '{udf:Samplesheet Format}' == 'V2' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Samplesheet_v2.csv'; \
elif[ '{udf:Reverse Complement Workflow}' == 'true' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Reverse_Complement_Samplesheet.csv'; \
elif [ '{udf:Reverse Complement Workflow}' == 'false' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Samplesheet.csv'; \
else echo 'UDF Reverse Complement Workflow is empty or undefined.'; exit -1; fi
\
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/$templateFilename \
-o {compoundOutputFileLuid0}.csv \
```


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```
-q true \
-destLIMSID {compoundOutputFileLuid0} \
-l {compoundOutputFileLuid1}"
```

4. Select Save.

Update the Validate Run Setup and Generate Sample Sheet automation on the Load to Flowcell (NovaSeq 6000 v3.2) step as follows.

1. From Configuration, select the Automation tab.
2. Under the Step Automation tab, select the Validate Run Setup and Generate Sample Sheet automation enabled on the Under the Load to Flowcell (NovaSeq 6000 v3.2) step.
3. Update the existing command line as follows.
   Changes to the existing command line are in red.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-exp '{udf:Validation Script}' \
-log {compoundOutputFileLuid1} \
-t true \
&& if [ '{udf:Samplesheet Format}' == 'V2' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Samplesheet_v2.csv'; \
elif[ '{udf:Reverse Complement Workflow}' == 'true' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Reverse_Complement_Samplesheet.csv'; \
elif [ '{udf:Reverse Complement Workflow}' == 'false' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Samplesheet.csv'; \
else echo 'UDF Reverse Complement Workflow is empty or undefined.'; exit -1; fi
\
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/$templateFilename \
-o {compoundOutputFileLuid0}.csv \
-q true \
-destLIMSID {compoundOutputFileLuid0} \
-l {compoundOutputFileLuid1}"
```

4. Select Save.

Add New v2 Sample Sheet Template File

To add the new sample sheet template file, download `NovaSeq_BCL2FASTQ_Samplesheet_v2.csv` and place the file in the following location:

`/opt/gls/clarity/extensions/conf/driverfiletemplates`

Modify Step to Display New Custom Fields

1. From Configuration, select the Lab Work tab.
2. In the Master Steps list, select the Denature and Dilute (NovaSeq 6000 v3.2) master step.
3. Select the Record Details milestone.
4. In Master Step Fields, add the new custom fields.
   - Analysis Software Version
   - Override Cycles
   - Samplesheet Format
5. Select the check box and save the step.
6. Repeat steps 1 - 5 for the Load to Flowcell (NovaSeq 6000 v3.2) step.

NovaSeq 6000 Integration v3.4.0 Release Notes

These Release Notes describe the key changes to software components for the Clarity LIMS NovaSeq 6000 Integration Package since version 3.3.

This is an optional software update for customers interested in the features available with this release.

If upgrading from a version prior to version 3.3, review the release notes for v3.3 for a list of features and bug fixes introduced in that version.

Compatibility

This version of the integration package is compatible with the following software:

- Clarity LIMS v5.1 and later
- NGS Extensions Package v5.21 and later
- Illumina Preset Protocols (IPP) v2.3.0 and later
- NovaSeq Control Software (NVCS) v1.8.0
- Illumina bcl2fastq v2.20

New Features

Features provided with this release of the integration package are as follows.

- Support for Clarity LIMS v6.0. Clarity LIMS v4.x and v5.0 are not supported.
- Support for NVCS v1.8.0 and v2 sample sheets.
- Updated NovaSeq 6000 v3.3 workflow installed from IPP v2.3.0.

- Tech refresh to upgrade third party libraries.
- Fixes to address the following Apache log4j vulnerabilities:
  - CVE-2021-44228
  - CVE-2021-45046
  - CVE-2021-45105
  - CVE-2021-44832
- Security fixes.

Defect Repairs

N/A

Known Issues

Blocker alert: When an artifact is removed and queued to another workflow, the "Queued" workflow-stage of the artifact is missing on the API level. Consequently, the recipe request of a NovaSeq run fails due to the missing "Queued" stage.

This issue only affects Clarity LIMS v5.2.1. The issue will be fixed in the upcoming Clarity LIMS v5.2.2 release.

NovaSeq 6000 Integration v3.4.0 Sample Sheet Customization

Clarity LIMS NovaSeq 6000 v3.4 Integration Package, together with Illumina Preset Protocols v2.3, provides sample sheet v2 generation functionality. This new functionality allows for ICA support.

The default configuration generates a sample sheet that contains content used for sequencing and BCL Convert. You can also generate v2 sample sheets that include custom content by editing the `NovaSeq_BCL2FASTQ_Samplesheet_v2.csv` template (included with IPP v2.3 and the NovaSeq 6000 v3.3 workflow). For example, custom content might include settings and data for other analysis applications, or additional settings used for BCL conversion.

Depending on the information required by the analysis software, you might need to include additional sample data tables in the generated sample sheet. If you need help determining the content to include in a sample sheet file, consult with the Illumina Support team.

Complete the following steps to create a customized v2 sample sheet template:

1. Create a custom sample sheet template file by editing the default template settings.
2. Create a custom sample sheet template file for analysis.
3. Upload the custom template file to Clarity LIMS.
4. Modify the automation to generate a sample sheet using the custom template file.

For information on troubleshooting the template file and its associated automation, refer to *Troubleshooting Template File Generator* on page 2485.

Edit Default Template File Settings

In this example, two additional settings (AdapterRead1 and AdapterRead2), with predefined values, are added to the BCLConvert_Settings section of the `NovaSeq_BCL2FASTQ_Samplesheet_v2.csv` template file.

Additionally, the settings are configured to retrieve values from the Software Version and Override Cycles master step fields.

After editing, the BLConvert_Settings section appears as follows.

```
[BCLConvert_Settings]
SoftwareVersion,${PROCESS.UDF.Analysis Software Version##Value}
OverrideCycles,${PROCESS.UDF.Override Cycles##Value}
AdapterRead1,CTGTCTCTTATACACATCT
AdapterRead2,GCTAGCTAGCTAGCAGCTT
```

Naming the Output File

The following line in the default template tells the driver_file_generator script to use the OUTPUT.CONTAINER.NAME format for the name of the output file:

```
OUTPUT.FILE.NAME,${OUTPUT.CONTAINER.NAME}.csv
```

As the automation does not have the context for this name value, remove this line from the file and use the driver_file_generator -o option to set the output file name. For more information and an example of -o option usage, refer to the Modify Automation Script to Generate a Custom Sample Sheet section.

Naming the Template File

After editing the default template, save it with a new file name (eg, `NovaSeq_BCL2FASTQ_Samplesheet_v2-modified.csv`).

The example template file is shown in the following drop-down section.

Modified Template Example

```
SORT.BY.${INPUT.CONTAINER.ROW}${INPUT.CONTAINER.COLUMN}
PROCESS.POOLED.ARTIFACTS
CONTROL.SAMPLE.DEFAULT.PROJECT.NAME,Controls
HIDE, ${PROCESS.UDF.Read 2 Cycles##Value}, IF, NODATA
HIDE, ${PROCESS.UDF.Index Read 1##Value}, IF, NODATA
HIDE, ${PROCESS.UDF.Index Read 2##Value}, IF, NODATA
HIDE, ${PROCESS.UDF.Analysis Software Version##Value}, IF, NODATA
HIDE, ${PROCESS.UDF.Override Cycles##Value}, IF, NODATA
HIDE, ${INPUT.REAGENT.SEQUENCE##Dual}, IF, NODATA
<HEADER_BLOCK>
```

```
[Header]
FileFormatVerion,2
RunName,${PROCESS.UDF.Experiment Name}
InstrumentPlatform,NovaSeq
InstrumentType,NovaSeq 6000
IndexOrientation,Forward

[Reads]
Read1Cycles,${PROCESS.UDF.Read 1 Cycles}
Read2Cycles,${PROCESS.UDF.Read 2 Cycles##Value}
Index1Cycles,${PROCESS.UDF.Index Read 1##Value}
Index2Cycles,${PROCESS.UDF.Index Read 2##Value}

[BCLConvert_Settings]
SoftwareVersion,${PROCESS.UDF.Analysis Software Version##Value}
OverrideCycles,${PROCESS.UDF.Override Cycles##Value}
AdapterRead1,CTGTCTCTTATACACATCT
AdapterRead2,GCTAGCTAGCTAGCAGCTT

[BCLConvert_Data]
</HEADER_BLOCK>
<HEADER>
Sample_ID,Index,Index2
</HEADER>
<DATA>
${SAMPLE.NAME##NoSpecialCharacters},${INPUT.REAGENT.SEQUENCE##Single},${INPUT.RE
AGENT.SEQUENCE##Dual}
</DATA>

<TOKEN_FORMAT>
${INPUT.REAGENT.SEQUENCE##Single}
def index = token.indexOf("-")
if (index > 0) {
return token.substring(0,index)
} else {
return token
}

${INPUT.REAGENT.SEQUENCE##Dual}
```


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```
def index = token.indexOf("-")
if (index >= 0) {
return token.substring(index + 1)
} else {
return null
}

${SAMPLE.NAME##NoSpecialCharacters}
return token.replaceAll(\"[^a-zA-Z0-9_]\", \"_\")

${PROCESS.UDF.Read 2 Cycles##Value}
if (token == '0') {
return null
}
return token

${PROCESS.UDF.Index Read 1##Value}
if (token == '0') {
return null
}
return token

${PROCESS.UDF.Index Read 2##Value}
if (token == '0') {
return null
}
return token

${PROCESS.UDF.Analysis Software Version##Value}
if (token == '0') {
return null
}
return token

${PROCESS.UDF.Override Cycles##Value}
if (token == '0') {
return null
}
return token
```


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```
</TOKEN_FORMAT>
```

Create Custom Template File for Analysis

When using a template file for analysis, the file must include a Sample data section containing HEADER and DATA blocks. For information on creating HEADER and DATA blocks, refer to *Creating Template Files* on page 2454.

Each template file can have only one Sample data section containing these blocks. If more Sample data tables are needed, create multiple template files and merge them using the concatenation command (`cat`) in the automation script. An example script that uses this command is provided in the Modify Automation Script to Generate a Custom Sample Sheet section.

Following is an example of a template file that includes HEADER and DATA blocks. The two empty lines after `<HEADER_BLOCK>` provide proper spacing when merging multiple files.

```
<HEADER_BLOCK>


[DragenGermline_Settings]
SoftwareVersion,3.8.4
ReferenceGenomeDir,${PROCESS.UDF.Reference Genome Directory}
MapAlignOutFormat,true
KeepFastq,true

[DragenGermline_Data]
</HEADER_BLOCK>

<HEADER>
Sample_ID
</HEADER>

<DATA>
${SAMPLE.NAME##NoSpecialCharacters}
</DATA>
```

- The [DragenGermline_Settings] section provides ICA with information about the analysis application. It tells ICA that this section belongs to the Dragen Germline Analysis Application and allows for the extraction of the settings information required by the analysis run.
- The sample DATA section provides ICA with information about the analysis application. It tells ICA that this section belongs to the Dragen Germline Analysis Application, and allows for the extraction of the sample data required by the analysis run.

Upload Custom Template File

1. From Configuration, select the Automation tab.
2. From the Automation tab, select the Step Automation tab.
3. In the automations list, select the automation to modify.
4. Under Template Files, select Upload File and upload the custom template file.

After uploading the template file, a new token for the file displays in the Tokens section. You can use this token in the automation command line, as described in the following section.

Modify the Automation to Generate a Custom Sample Sheet

After uploading a template file, modify the automation so that the driver_file_generator script uses the new file to generate the sample sheet. Following is an example of a modified automation command line.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} \
script:driver_file_generator \
-t {file:NovaSeq_BCL2FASTQ_Samplesheet_v2-modified.csv} \
-o 1.csv \
script:driver_file_generator \
-t {file:Custom_DrageGermline.csv} \
-o 2.csv \
&& cat 1.csv 2.csv > {compoundOutputFileLuid0}.csv"
```

In this example, the last line uses concatenation to merge the two files referenced in the script (eg, `NovaSeq_BCL2FASTQ_Samplesheet_v2-modified.csv` and `Custom_DrageGermline.csv`) into a single file.

NovaSeq 6000 Integration v3.3.0 Configuration and User Interaction

The Illumina NovaSeq 6000 Integration Package v3.3.0 supports the integration of Clarity LIMS to Illumina NovaSeq 6000 instruments.

Note the following key changes for this version of the integration:

- Support for Secret Management mechanism
- A workflow as part of the Preconfigured Workflows Package (PCWP) v2.0.1 and Illumina Preset Protocol (IPP) v2.0

This version includes the following configuration change:

- Support for PhiX spike-in

This User and Configuration Guide describes the integration and the preconfigured workflows, protocols, steps, and automations. The guide also lists installed components and provides configuration requirements, rules, and constraints.

For instructions on validating and troubleshooting the Illumina NovaSeq 6000 Integration, see the *NovaSeq 6000 Integration v3.3.0 Validation and Troubleshooting Guide*.

The configuration provided in this integration has been established to support NovaSeq 6000 lab processes. Any configuration changes to protocols or workflows (including renaming protocols, steps, and fields) could break process.

Integration Overview

The Illumina NovaSeq 6000 Integration Package v3.3.0 integration includes the following items:

- Preconfigured NovaSeq 6000 v3.1 workflow that maps to lab protocols and instrument runs.
- Preconfigured protocols:
  - Run Format (NovaSeq 6000 v3.1)
  - NovaSeq Standard (NovaSeq 6000 v3.1) (supports the loading of pooled libraries into a library tube)
  - NovaSeq Xp (NovaSeq 6000 v3.1) (supports individual lane loading on the NovaSeq)
  - AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.1)
- Automated generation of a sample sheet for use with bcl2fastq2 v2.20 analysis software. This file is automatically uploaded to the instrument via the Sequencer API and is used to set up the run.
- Automatic validation of run setup information. This information is used to create the run recipe and initiate the run.
- Automated tracking of the NovaSeq sequencing run and parsing of run statistics into Clarity LIMS, including:
  - Progress and metrics of sequencing run
  - Per-instrument sequencing runs (tracked as part of the Run ID field value)
  - Sequencing run parameters
  - Sequencing run data directory location
  - Real-Time Analysis v3 (RTA3) version and other run-specific information
- [Optional] Preconfigured Library Prep Validation v1.1.0/v2.0.1 workflow used for validation purposes only. The workflow contains a single-step protocol that models the library prep required to produce normalized libraries that are ready for the NovaSeq 6000 v3.1 workflow. See the *NovaSeq 6000 Integration v3.3.0 Validation and Troubleshooting Guide*.
  - For Clarity LIMS v4.2 and v4.3 users, Library Prep Validation v1.1.1 workflow is available in PCWP v2.0.1 Package.

  - For Clarity LIMS v5.1 and above users, Library Prep Validation v2.0.1 workflow is available in IPP v2.0 Package.
- The above two versions of Library Prep Validation workflow are identical. To avoid confusion, we only show v2.0.1 as an example in this documentation.

  The term 'UDF' (user-defined field) is used in Clarity LIMS v4.x. The term 'custom fields' is used in Clarity LIMS v5.x. The terms are used interchangeably and referred to in this guide as custom fields.

Prerequisites and Assumptions

It is assumed that samples enter the NovaSeq 6000 v3.1 workflow as normalized libraries. That is, before they are assigned to the workflow:

- Samples have been accessioned into the Clarity LIMS.
- Samples have been run through QC and library prep.
- Samples have been normalized, and the value is captured in a field called Normalized Molarity (nM).

Workflows, Protocols, and Steps

The Illumina NovaSeq 6000 Integration Package v3.2.2 includes two workflows:

- NovaSeq 6000 v3.1
- Library Prep Validation v2.0.1 (optional, but recommended for validation purposes)

The following table describes the protocols and steps included in these workflows.

Table 177 Illumina NovaSeq 6000 Integration Package v3.3.0 Protocols and Steps

Library Prep Validation v2.0.1 Workflow		
Protocol Name	Protocol Purpose	Steps Included in Protocol
Library Prep Validation v2.0.1	Included for validation purposes only, this protocol models the library prep steps required to advance samples to the Run Format (NovaSeq 6000 v3.3.0) protocol. The protocol contains a single step - Library Prep Validation v2.0.1. After this step, a routing script sends the samples to the first step of the NovaSeq 6000 v3.1 workflow - Define Run Format (NovaSeq 6000 v3.1).	• Library Prep Validation v2.0.1
NovaSeq 6000 v3.0 Workflow		
Protocol Name	Protocol Purpose	Steps Included in Protocol
Run Format (NovaSeq 6000 v3.1)	Allows for the assignment of per sample values for Loading Workflow Type, Normalized Molarity, Flowcell Type, and Final Loading Concentration (pM): • Loading Workflow Type: Select from NovaSeq Standard or NovaSeq Xp. • Normalized Molarity: Enter a value for each sample. • Flowcell Type: Select from options SP, S1, S2, or S4. • Final Loading Concentration (pM): Select from options 225 (PCR-free workflows) or 400 (Nano workflows), or enter a different value. Compares each sample Normalized Molarity value with the Minimum Molarity value. Routing script sends samples to the NovaSeq Standard or NovaSeq Xp protocol, according to the selected Loading Workflow Type. Samples with Normalized Molarity less than Minimum Molarity are removed from the workflow.	• Define Run Format (NovaSeq 6000 v3.1)

Library Prep Validation v2.0.1 Workflow		
Protocol Name	Protocol Purpose	Steps Included in Protocol
NovaSeq 6000 v3.0 Workflow		
Protocol Name	Protocol Purpose	Steps Included in Protocol
NovaSeq Standard (NovaSeq 6000 v3.1)	Samples are pooled and added to the library tube in preparation for the NovaSeq run. Run setup information is validated and sample sheet is generated. Routing script sends library tube to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.1) protocol.	1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.1) 2. Dilute and Denature (NovaSeq 6000 v3.1)
NovaSeq Xp (NovaSeq 6000 v3.1)	Samples are pooled and added to lanes on the NovaSeq flow cell. The flow cell type is determined by the option selected in the Define Run Format (NovaSeq 6000 v3.1) step. Run setup information is validated and sample sheet is generated. Routing script sends flow cell to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.1) protocol.	1. Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.1) 2. Dilute, Denature, and ExAmp (NovaSeq 6000 v3.1) 3. Load to Flowcell (NovaSeq 6000 v3.1)
AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.1)	All samples complete the workflow by going through this protocol. This protocol contains one fully automated step. Do not add samples to the Ice Bucket or start the step. The integration starts the step automatically.	• AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.1)

Validation Workflow

The Library Prep Validation v2.0.1 workflow allows for validation of the system after installation is complete. For details, see the *NovaSeq 6000 Integration v3.3.0 Validation and Troubleshooting Guide*.

Step Automation

The following table lists the step automation included in the NovaSeq 6000 v3.1 workflow. Automation details are provided in the sections following the table.

See *NovaSeq Integration v3.3.0 Validation and Troubleshooting Guide* for details on the validation workflow,

Table 178 NovaSeq 6000 v3.1 workflow step automations

Protocol	Step	Automation
1. Run Format (NovaSeq 6000 v3.1)	• Define Run Format (NovaSeq 6000 v3.1)	• Routing Script • Set Next Steps
2. NovaSeq Standard (NovaSeq 6000 v3.1)	1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.1)	• Calculate Volumes • Set Next Step • Validate Inputs Flowcell Type and Single Pool • Validate Unique Indexes (Not used. This functionality is handled by the core Clarity LIMS configuration for pooling.)
	2. Dilute and Denature (NovaSeq 6000 v3.1)	• Routing Script • Check Library Tube Barcode • Validate Run Setup and Generate Sample Sheet • Validate Single Input
3. NovaSeq Xp (NovaSeq 6000 v3.1)	1. Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.1)	• Calculate Volumes • Set Next Step • Validate Inputs Flowcell Type and Single Pool • Validate Unique Indexes (Not used. This functionality is handled by the core Clarity LIMS configuration for pooling.)
	2. Dilute, Denature, and ExAmp (NovaSeq 6000 v3.1)	• Calculate Volumes • Set Next Step • Validate Inputs Flowcell Type
	3. Load to Flowcell (NovaSeq 6000 v3.1)	• Validate Flowcell Barcode • Validate Inputs and Selected Container • Validate Run Setup and Generate Sample Sheet

Protocol	Step	Automation
4. AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.1)	• AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.1)	• Update Lane Number • Set Next Steps (Not used. By default, this functionality is determined by the Sequencer API. If necessary, enable the automation and use it to override the next step behavior used by the Sequencer API.)

Configuration Details

The following sections describe the configuration installed with the Illumina NovaSeq 6000 Integration.

The Illumina NovaSeq 6000 Integration Package v3.3.0 supports Clarity LIMS v4 (4.2 and later), and v5 (5.3 and later).

As of Clarity LIMS v5, the term user-defined field (UDF) has been replaced with custom field in the user interface (the API resource is still called UDF). There are two types of custom fields:

- Master Step Fields—Configured on master steps. These fields only apply to the master step on which they are configured and the steps derived from those master steps.
- Global Fields—Configured on entities such as submitted sample, derived sample, and measurement. These fields apply to the whole Clarity LIMS system.

Protocol 1: Run Format (NovaSeq 6000 v3.1)

This protocol sets the Loading Workflow Type and allows the choice of the appropriate Flowcell Type and Final Loading Concentration (pM). After the protocol, a routing script sends the normalized libraries to either the NovaSeq Standard (NovaSeq 6000 v3.1) or the NovaSeq Xp (NovaSeq 6000 v3.1) protocol.

This protocol contains one step: Define Run Format (NovaSeq 6000 v3.1). The step is described in the following section.

Step 1. Define Run Format (NovaSeq 6000 v3.1)

Step input: NTP (normalized libraries)

Step output: None

The following automations are configured on this step:

- Routing Script
- Set Next Steps

Run Format (NovaSeq 6000 v3.1) automation configuration is described in the following section, in the order in which the automations are triggered at run time.

Set Next Steps

Automatically triggered on exit of the Record Details screen, this automation completes the following actions:

- Sets the next step for samples to REMOVE:

```
nextStep = ::REMOVE::
```

- Calculates the Minimum Molarity using the following formula:

```
input.::Minimum Molarity (nM):: = (5 * input.::Final Loading Concentration
(pM)::)/1000
```

- Checks Normalized Molarity value. For samples with no Normalized Molarity value (ie, empty value, not including 0), it generates an error message informing that the field cannot be empty:

```
if (!input.hasValue(::Normalized Molarity (nM)::)) {
  fail(::The Normalized Molarity cannot be empty.::) ;
}
```

- Compares each sample Normalized Molarity value with the Minimum Molarity value. If Normalized Molarity value is lower than the Minimum Molarity value, it sets the samples Loading Workflow Type to [Remove from workflow] and records a message in the Warning field for the sample:

```
else if (input.::Normalized Molarity (nM):: < input.::Minimum Molarity (nM)::) {
  input.::Warning:: = ::The Normalized Molarity is too low.:: ;
  input.::Loading Workflow Type:: = ::[Remove from workflow]:: ; }
else { input.::Warning:: = ::not applicable:: }
```

At this point there are two options:

- Correct the Normalized Molarity value on the Record Details screen. Edit the Loading Workflow Type field and set it to NovaSeq Standard or NovaSeq Xp, as applicable.
- Complete the protocol without correcting the Normalized Molarity value. In this case, those samples are removed from the workflow.

Routing Script

Automatically triggered on exit of the step, this automation invokes the changeWorkflow script, which routes step inputs appropriately.

- Samples with Loading Workflow Type field value = NovaSeq Standard are routed to the NovaSeq 6000 v3.1 workflow and queued for the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.1) step.
- Samples with Loading Workflow Type field value = NovaSeq Xp are routed to the NovaSeq 6000 v3.1 workflow and queued for the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.1) step.

Default automation command line is as follows:

```
bash -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar
-u {username} -p {password} -i {stepURI:v2} -l {compoundOutputFileLuid0}
script:changeWorkflow \
\
--FIELD_NAME 'Loading Workflow Type' \
--FIELD_VALUE 'NovaSeq Standard' \
--WORKFLOW 'NovaSeq 6000 v3.1' \
--STEP 'Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.1)' \
--INPUTS_OR_OUTPUTS 'INPUTS' \
\
--FIELD_NAME 'Loading Workflow Type' \
--FIELD_VALUE 'NovaSeq Xp' \--WORKFLOW 'NovaSeq 6000 v3.1' \
--STEP 'Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.1)' \
--INPUTS_OR_OUTPUTS 'INPUTS'"
```

Step UDFs/Custom Fields

There are four fields defined on the Define Run Format (NovaSeq 6000 v3.1) step.

The following table lists field configuration details.

Table 179 Define Run Format (NovaSeq 6000 v3.1) Step Custom Field Configuration

Field Name	Field Type	Field Constraints	Preset Values/Additional Options and Dropdown Items
Comment	Multiline Text	• Required: No • Read Only: No	None
Flowcell Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• SP • S1 • S2 • S4
Instruction	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	• Default: Add Flowcell Type and Loading Workflow Type below
Loading Workflow Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp

Global Custom Fields

The following table lists the global custom fields that are configured to display on the Define Run Format (NovaSeq 6000 v3.1) step.

Table 180 Global Custom Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Configured on Analyte in Clarity LIMS v4 and Derived Sample in Clarity LIMS v5:			
Adjusted Per Sample Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Final Loading Concentration (pM)	Numeric (v4) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 225 • 400 • Decimal Places Displayed: 0
Flowcell Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• SP • S1 • S2 • S4
Loading Workflow Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
Minimum Molarity (nM)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2
Normalized Molarity (nM)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2
Per Sample Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Warning	Single-line Text (v4) Text Dropdown (v5)	• Required: No • Read Only: Yes • Custom Entries: Yes	• The Normalized Molarity (nM) is too low. • Not applicable

User Interaction

1. On the Record Details screen, complete the following fields in the Sample Details table (values can vary across samples):
   - Loading Workflow Type: Select NovaSeq Standard or NovaSeq Xp from the drop-down list.
   - Flowcell Type: Select SP, S1, S2, or S4 from the drop-down list.
   - Final Loading Concentration (pM): Select from the two preset options: 225 (for PCR-free workflows) or 400 (for Nano workflows). Or enter a different value.
   - Normalized Molarity (nM): These values are copied over from the previous step. If not populated during library prep, enter the values here.
   - Select Next Steps. This action triggers the Next Steps automation, which completes the following actions:
     - Sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to successfully advance samples to the next step.
     - Calculates the Minimum Molarity.
     - Checks Normalized Molarity value. For samples with no Normalized Molarity value (ie, empty value, not including 0), generates an error message informing that the field cannot be empty.
     - Compares each sample Normalized Molarity value with the Minimum Molarity value.
2. On the Assign Next Steps screen, note the following items:
   - In the Sample Details table, the Next Step for all samples is populated with Remove from workflow, regardless of the Loading Workflow Type.

     Do not change this value. If Next Step is not set to Remove from workflow, the routing script cannot route samples correctly.

   - For samples where Normalized Molarity is lower than the Minimum Molarity value, the Loading Workflow Type is set to Remove from workflow. A message is recorded in the Warning field for the sample:

   At this point there are two options:
   - Return to the Record Details screen and adjust the Normalized Molarity value so that it equals or exceeds the Minimum Molarity value. Set the Loading Workflow Type to NovaSeq Standard.
   - Complete the protocol without correcting the Normalized Molarity value. In this case, the samples in question are removed from the Clarity LIMS workflow.
3. Select Finish Step. The Routing Script automation is triggered:
   - Samples whose Loading Workflow Type is set to Remove from workflow (ie, where Normalized Molarity value is lower than the Minimum Molarity) are removed from the Clarity LIMS workflow.

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- Samples whose Loading Workflow Type is set to NovaSeq Standard are routed to the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.1) step. The first of two steps in the NovaSeq Standard (NovaSeq 6000 v3.1) protocol.
- Samples whose Loading Workflow Type is set to NovaSeq Xp are routed to the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.1) step. The first of three steps in the NovaSeq Xp (NovaSeq 6000 v3.1) protocol.

Protocol 2: NovaSeq Standard (NovaSeq 6000 v3.1)

Samples are routed to this protocol if their Loading Workflow Type value is set to NovaSeq Standard. Samples are pooled and added to a library tube in preparation for the NovaSeq run.

At the end of this protocol, a routing script sends the library tube to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.1) protocol.

This protocol contains two steps:

- Step 1: Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.1)
- Step 2: Dilute and Denature (NovaSeq 6000 v3.1)

Step 1: Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.1)

In this step, place libraries into a single pool, manually. Resuspension buffer and reagents are added.

Only create one pool per step.

Step input: NTP (normalized libraries)

Step output: Bulk pool

The following automations are configured on the step:

- Calculate Volumes
- Set Next Step
- Validate Inputs Flowcell Type and Single Pool
- Validate Unique Indexes (Not used. This functionality is handled by the core Clarity LIMS configuration for pooling.)

Validate Inputs Flowcell Type and Single Pool

Automatically triggered on exit of the Pooling screen, this automation completes the following actions:

- Checks that all samples in the pool have the same Flowcell Type assigned to them.
- Checks that only one pool has been created.
- Changes the value of error and logging messages to reference the type of pool passed as bulk pool.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar \
```

```
script:validate_flowcell_for_input_pools \
  -i {stepURI:v2} \
  -u {username} \
  -p {password} \
  -l {compoundOutputFileLuid1} \
  -validateSingleOutput true \
  -poolType bulk"
```

Calculate Volumes

Automatically triggered when selecting the Calculate Volumes button on the Record Details screen, this automation completes the following actions:

- Calculates the number of samples in the pool.

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: + 1
```

- Sets the value of the Bulk Pool Volume (µl), and PhiX Volume (µl) field based on the selected Flowcell Type.

```
step.::PhiX Volume (µl):: = !step.hasValue(::% PhiX (2.5nM) Spike-In::) ? 0 :
step.::% PhiX (2.5nM) Spike-In::;
if (input.::Flowcell Type:: == ::SP::) {
step.::Bulk Pool Volume (µl):: = step.::Number of Flowcells to Sequence:: * 100
+ 30;
step.::PhiX Volume (µl):: *= 0.8
};
if (input.::Flowcell Type:: == ::S1::) {
step.::Bulk Pool Volume (µl):: = step.::Number of Flowcells to Sequence:: * 100
+ 30;
step.::PhiX Volume (µl):: *= 0.8
};
if (input.::Flowcell Type:: == ::S2::) {
step.::Bulk Pool Volume (µl):: = step.::Number of Flowcells to Sequence:: * 150
+ 30;
step.::PhiX Volume (µl):: *= 1.1
};
if (input.::Flowcell Type:: == ::S4::) {
step.::Bulk Pool Volume (µl):: = step.::Number of Flowcells to Sequence:: * 310
+ 30;
step.::PhiX Volume (µl):: *= 2.1
};
if (step.::PhiX Volume (µl):: == 0) {
step.::PhiX Volume (µl):: = ::::
```

```
};
```

- Calculates the Per Sample Volume (µl) to be added to the pool:

```
input.::Per Sample Volume (µl):: = ( ( (input.::Final Loading Concentration
(pM):: * 5 / 1000) / input.::Normalized Molarity (nM):: ) * step.::Bulk Pool
Volume (µl):: ) / step.::Number of Samples in Pool::
```

> To ensure accurate pipetting of each sample in a pool for sequencing, the Per Sample Volume (µl) value must be equal to or higher than the Minimum Per Sample Volume (µl) (default value, set at 5, may be edited).
>
> Assuming the default Minimum Per Sample Volume (µl) value of 5, completes the following steps for a given batch:
>
> 1. If the smallest Per Sample Volume (µl) value is less than 5, automatically assigns a value of 5 to the sample Adjusted Per Sample Volume (µl) field.
> 2. Adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

- Calculates the Total Sample Volume (µl) field value:

```
step.::Total Sample Volume (µl):: = step.::Total Sample Volume (µl):: +
input.::Adjusted Per Sample Volume (µl)::
```

- If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) field value:

```
if (step.::Total Sample Volume (µl):: >= step.::Bulk Pool Volume (µl)::)
{output.::RSB Volume (µl):: = 0}
else {output.::RSB Volume (µl):: = step.::Bulk Pool Volume (µl):: - step.::Total
Sample Volume (µl)::} ;
```

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ;
output.::Loading Workflow Type:: = input.::Loading Workflow Type:: ;
```

- Sets the Volume of Pool to Denature (µl) value and calculates NaOH Volume (µl) and Tris-HCl Volume (µl) values, based on the Flowcell Type:

```
if ( input.::Flowcell Type:: == ::SP:: ) { output.::Volume of Pool to Denature
(µl):: = 100 ; output.::NaOH Volume (µl):: = 25 ; output.::Tris-HCl Volume
(µl):: = 25 } ;
if ( input.::Flowcell Type:: == ::S1:: ) { output.::Volume of Pool to Denature
(µl):: = 100 ; output.::NaOH Volume (µl):: = 25 ; output.::Tris-HCl Volume
(µl):: = 25 } ;
if ( input.::Flowcell Type:: == ::S2:: ) {output.::Volume of Pool to Denature
(µl):: = 150 ; output.::NaOH Volume (µl):: = 37.50 ; output.::Tris-HCl Volume
(µl):: = 37.50 } ;
```

```
if ( input.::Flowcell Type:: == ::S4:: ) { output.::Volume of Pool to Denature
(µl):: = 310 ; output.::NaOH Volume (µl):: = 77.50 ; output.::Tris-HCl Volume
(µl):: = 77.50 }
```

- Uses the NovaSeq_Standard_Bulk_Pool1.csv, NovaSeq_Standard_Bulk_Pool2.csv, and NovaSeq_Standard_Bulk_Pool3.csv template files to generate a single CSV file containing information about the pool and the samples it contains. The generated file is available for download on the Step Setup screen of Dilute and Denature (NovaSeq 6000 v3.1).

```
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} \ script:driver_file_generator \
  -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Standard_Bulk_
Pool1.csv \
  -o 1.csv \ script:driver_file_generator \
  -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Standard_Bulk_
Pool2.csv \
  -o 2.csv \ script:driver_file_generator \
  -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Standard_Bulk_
Pool3.csv \
  -o 3.csv \
&& cat 1.csv 2.csv 3.csv > {compoundOutputFileLuid0}.csv
```

Set Next Step

Automatically triggered on exit of the Record Details screen, this automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol, Dilute, and Denature (NovaSeq 6000 v3.1):

```
nextStep = ::ADVANCE::
```

Step UDFs/Custom Fields

There are seven fields defined on the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.1) step. The following table lists configuration details.

Table 181 Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.1) Step Custom Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
% PhiX (2.5 nM) Spike-In	Numeric	• Required: No • Read Only: No	• Range From: 1 to 10 • Decimal Places Displayed: 0
Bulk Pool Volume (µl) (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2
Number of Flowcells to Sequence	Numeric	• Required: Yes • Read Only: No	• Range From: 1 to 10 • Decimal Places Displayed: 0
Minimum Per Sample Volume (µl)	Numeric	• Required: Yes • Read Only: No	• Default: 5 • Decimal Places Displayed: 2
Number of Samples in Pool (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Default: 0 • Decimal Places Displayed: 0
PhiX Volume (µl) (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2
Total Sample Volume (µl) (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Default: 0 • Decimal Places Displayed: 2

Global Custom Fields

The following table lists the global custom fields that are configured to display on the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.1) step.

Table 182 Global Custom Fields Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Configured on Analyte in Clarity LIMS v4 and Derived Sample in Clarity LIMS v5:			
Flowcell Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• SP • S1 • S2 • S4
Loading Workflow Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
NaOH Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
RSB Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Tris-HCl Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Volume of Pool to Denature (µl) Used in Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.1) step only. Displays on Record Details screen and in the generated CSV file.	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0

User Interaction

1. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucket, and select View Ice Bucket.
2. On the Ice Bucket screen, select Begin Work.
3. On the Pooling screen complete the following actions:
   a. Create a pool by dragging samples into the Pool Creator.
   b. Name the pool or accept the default name (Pool #1).

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c. Select Record Details.
On exiting the Pooling screen, the Validate Inputs Flowcell Type and Single Pool is triggered. The automation checks that all samples in the pool are assigned the same Flowcell Type, and only one pool is created.

4. On the Record Details screen in the Step Details area, complete the two required fields and one optional field, as needed:
   - Number of Flowcells to Sequence—This value is used in volume calculations, to make sure that volumes are sufficient for the number of times the pool is sequenced.
   - Minimum Per Sample Volume (µl)—In many applications, and particularly where robotics are used, a minimum sample volume is required. This value is used to calculate how much of each sample is included in the pool.
   - % PhiX (2.5 nM) Spike-In—This value is used to calculate the volume of PhiX v3 control to include in the pool for the given percentage of spike-in. The field is optional and may be edited.
5. In the Sample Details table, select the pool icon to view details on the pool composition.
6. Select Calculate Volumes. This action triggers the Calculate Volumes automation, which completes volume calculations based on the selected Flowcell Type:
   - Calculates Bulk Pool Volume (µl) value.
   - Calculates PhiX Volume (µl) value if % PhiX (2.5 nM) Spike-In has value.
   - Calculates the Per Sample Volume (µl) value to be added to the pool.
   - Calculates the Total Sample Volume (µl) value.
   - If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) value.
   - Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
   - Populates the Volume of Pool to Denature (µl), NaOH Volume (µl) and T ris-HCl Volume (µl) columns of the Sample Details table. (Values are set by a script and are not editable.)
   - Generates the Calculation File (CSV) and attaches it to the step. This file contains volume information about the pool and the individual samples it contains. Select the file to download it, and then open it in Excel.
7. Select Next Steps. The Set Next Step automation is triggered. This automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol, Dilute, and Denature (NovaSeq 6000 v3.1).
   On the Assign Next Steps screen, the next step is already set to Dilute and Denature (NovaSeq 6000 v3.1).
8. Select Finish Step.
   At the end of this step, the pool of samples automatically advances to the Dilute and Denature (NovaSeq 6000 v3.1) step.

Step 2: Dilute and Denature (NovaSeq 6000 v3.1)

In this step, pooled samples are denatured and diluted by the addition of NaOH, Tris-HCl, and Resuspension Buffer (RSB). Manually place the pooled samples into the library tube for the NovaSeq run.

In addition, this step validates the run setup information and generates the sample sheet file.

Step input: Bulk pool

Step output: Library tube

The following automations are configured on the step:

- Routing Script
- Check Library Tube Barcode
- Validate Run Setup and Generate Sample Sheet
- Validate Single Input

These automations are described in the following sections, in the order in which they are triggered at run time.

Validate Single Input

Automatically triggered at the beginning of the step, this automation checks that there is only one container input to the step.

```
script:validateSampleCount -min 1 -max 1
```

Check Library Tube Barcode

Automatically triggered on exit of the Placement screen, this automation completes the following actions:

- Validates the library tube barcode to ensure it conforms to the barcode mask [A-Za-z]{2}[0–9]{7}-[A-Za-z]{3}:

```
if (!output.container.name.matches(::[A-Za-z]{2}[0-9]{7}-[A-Za-z]{3}::)) {
fail(::Invalid Library Tube Barcode. Verify and try again.::)
}
```

- Copies Flowcell Type and Loading Workflow Type field values from step inputs to outputs:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ;
output.::Loading Workflow Type:: = input.::Loading Workflow Type::
```

Validate Run Setup and Generate Sample Sheet

Triggered by a button on the Record Details screen, this automation completes the following actions:

- Copies the Flowcell Type from the step input to the Run Mode field (hidden):

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```
step.::Run Mode:: = input.::Flowcell Type::
```

- Validates the parameters entered on the Record Details screen, which is used to set up the run and generate the sample sheet file. See *Respond to Validation/Recipe Request Call from Instrument* for more details.
  - Experiment Name may only contain alphanumeric, dash, or underscore characters. Spaces are not permitted.
  - When Workflow Type = No Index, Index Read 1 must be zero.
  - When Workflow Type = Single Index, Index Read 1 must be greater than zero, and Index Read 2 must be zero.
  - When Workflow Type = Dual Index, both Index Read 1 and Index Read 2 must be greater than zero.

```
if(!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) {
fail(::Experiment Name contains prohibited characters. Allowed characters are:
a-z, A-Z, 0-9, -, and _::)
};
if (step.::Workflow Type:: == ::No Index::) {
if (step.::Index Read 1:: != 0 || step.::Index Read 2:: != 0) {
fail(::Index Read 1 and Index Read 2 must be 0 if No Index is selected.::)
}
} else if (step.::Workflow Type:: == ::Single Index::) {
if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) {
fail(::Index Read 1 must be greater than 0 and Index Read must be 0 if Single
Index is selected.::)
}
} else {
if (step.::Index Read 1:: == 0 || step.::Index Read 2:: == 0) {
fail(::Index Read 1 and Index Read 2 must be greater than 0 if Dual Index is
selected.::)
}
};
```

- Checks the Paired End and Read 2 Cycles field values.
  - If Paired End = True and Read 2 Cycles value is zero, generates an error.
  - If Paired End = False, Read 2 Cycles must be zero and UMI - Read 2 Length and UMI - Read 2 Start From Cycle must be empty.

```
if (step.::Paired End::.toBoolean()) {
if (step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) {
fail(::Read 1 Cycles and Read 2 Cycles must be greater than 0 if Paired End is
True.::)
}
```


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```
} else {
if (step.::Read 2 Cycles:: != 0) {
fail(::Read 2 Cycles must be 0 if Paired End is False.::)
};
if (step.hasValue(::UMI - Read 2 Length::) || step.hasValue(::UMI - Read 2 Start
From Cycle::)) {
fail(::UMI - Read 2 Length and UMI - Read 2 Start From Cycle cannot be defined
if Paired End is False.::)
}
};
```

- Checks the Flowcell field value.
  - If Flowcell Type value is not SP, checks that the value of both Read 1 Cycles and Read 2 Cycles is 151 or less. If value is greater than 151, generates an error message.

```
if (input.::Flowcell Type::!=::SP:: && step.::Read 1 Cycles:: > 151) {
fail(::Read 1 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
};
if (input.::Flowcell Type::!=::SP:: && step.::Read 2 Cycles:: > 151) {
fail(::Read 2 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
};
```

- Validates UMI Settings.
  - If UMI - Read 1 Length has value, then UMI - Read 1 Start From Cycle must have value.
  - If UMI - Read 1 Length has no value, then UMI - Read 1 Start From Cycle must not have value.
  - If UMI - Read 2 Length has value, then UMI - Read 2 Start From Cycle must have value.
  - If UMI - Read 2 Length has no value, then UMI - Read 2 Start From Cycle must not have value.
  - If UMI - Read 1 Length has no value, then UMI - Read 2 Length must not have value.
  - If UMI - Read 1 Start From Cycle has no value, then UMI - Read 2 Start From Cycle must not have value.

```
if (step.hasValue(::UMI - Read 1 Length::) && !step.hasValue(::UMI - Read 1
Start From Cycle::)) {
fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 1
Length is greater than 0.::)
};
if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 1
Start From Cycle::)) {
fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 1 Start From
Cycle is greater than 0.::)
};
```


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```
if (step.hasValue(::UMI - Read 2 Length::) && !step.hasValue(::UMI - Read 2
Start From Cycle::)) {
fail(::UMI - Read 2 Start From Cycle must be greater than 0 if UMI - Read 2
Length is greater than 0.::)
};
if (!step.hasValue(::UMI - Read 2 Length::) && step.hasValue(::UMI - Read 2
Start From Cycle::)) {
fail(::UMI - Read 2 Length must be greater than 0 if UMI - Read 2 Start From
Cycle is greater than 0.::)
};
if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 2
Length::)) {
fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 2 Length is
greater than 0.::)
};
if (!step.hasValue(::UMI - Read 1 Start From Cycle::) && step.hasValue(::UMI -
Read 2 Start From Cycle::)) {
fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 2
Start From Cycle is greater than 0.::)
};
```

- Outputs the Settings Header in sample sheet if UMI - Read 1 Length or UMI - Read 2 Length have value.

```
step.::Settings Header:: = step.hasValue(::UMI - Read 1 Length::) ||
step.hasValue(::UMI - Read 2 Length::) ? ::[Settings]:: : ::::;
```

- Sets the next step for samples to REMOVE:

```
nextStep = ::REMOVE::
```

- Generates the sample sheet and attaches it to the step. (See Sample Sheet Contents for more information.) Sample sheet template used depends on the selected value of Reverse Complement Workflow.

```
&& if [ '{udf:Reverse Complement Workflow}' == 'true' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Reverse_Complement_Samplesheet.csv'; \
elif [ '{udf:Reverse Complement Workflow}' == 'false' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Samplesheet.csv'; \
else echo 'UDF Reverse Complement Workflow is empty or undefined.'; exit -1; fi
\
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} \
script:driver_file_generator \
```

```
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/$templateFilename \
-o {compoundOutputFileLuid0}.csv \
-q true \
-destLIMSID {compoundOutputFileLuid0} \
-l {compoundOutputFileLuid1}"
```

Routing Script

Automatically triggered on exit of the step, this automation invokes the changeWorkflow script, which routes step outputs to the NovaSeq 6000 v3.1 workflow, and queues them for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.1) step.

Default automation command line is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar
-u {username} -p {password} -i {stepURI:v2} -l {compoundOutputFileLuid2}
script:changeWorkflow \
\
--FIELD_NAME 'N/A' \
--FIELD_VALUE 'N/A' \
--WORKFLOW 'NovaSeq 6000 v3.1' \
--STEP 'AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.1)' \
--INPUTS_OR_OUTPUTS 'OUTPUTS'"
```

Step UDFs/Custom Fields

There are 22 fields defined on the Dilute and Denature (NovaSeq 6000 v3.1) step. These fields are required for sample sheet and JSON file generation.

The following table lists field configuration details.

Table 183 Dilute and Denature (NovaSeq 6000 v3.1) Step Custom Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
BaseSpace Sequence Hub Configuration	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• Not Used • Run Monitoring Only • Run Monitoring and Storage

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Custom Recipe Path	Single-line Text (v4) Text Dropdown (v5)	• Required: No • Read Only: No	Not applicable
Experiment Name	Single-line Text (v4) Text (v5)	• Required: Yes • Read Only: No	Not applicable
Index Read 1	Numeric (v4) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 0 • 6 • 8 • Range From: 0 to 20 • Decimal Places Displayed: 0
Index Read 2	Numeric (v4) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 0 • 6 • 8 • Range From: 0 to 20 • Decimal Places Displayed: 0
Output Folder	Single-line Text (v4) Text (v5)	• Required: Yes • Read Only: No	Not applicable
Paired End	Single-line Text (v4) Text (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• True • False

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Read 1 Cycle	Numeric (v4) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 251* • 151 • 101 • 51 • Range From: 1 to 251 • Decimal Places Displayed: 0 *Value of 251 is only supported for SP flow cell type. For all other cell types, maximum value is 151.
Read 2 Cycle	Numeric (v4) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 251* • 151 • 101 • 51 • Range From: 1 to 251 • Decimal Places Displayed: 0 *Value of 251 is only supported for SP flow cell type. For all other cell types, maximum value is 151.
Reverse Complement Workflow	Check Box (v4) Toggle Switch (v5)	• Required: Yes • Read Only: No	• Default: Yes
Run Mode Not displayed in user interface)	Single-line Text (v4) Toggle Switch (v5)	• Required: No • Read Only: Yes • Custom Entries: No	• SP • S1 • S2 • S4
Sitting Header (not displayed in user interface)	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
UMI—Read 1 Length	Numeric (v4) Numeric (v5)	• Required: No • Custom Entries: Yes	Not applicable
UMI—Read 1 Start From Cycle	Numeric (v4) Numeric (v5)	• Required: No • Custom Entries: Yes	Not applicable
UMI—Read 2 Length	Numeric (v4) Numeric (v5)	• Required: No • Custom Entries: Yes	Not applicable
UMI—Read 2 Start From Cycle	Numeric (v4) Numeric (v5)	• Required: No • Custom Entries: Yes	Not applicable
Use Custom Index Read 1 Primer	Check Box(v4) Toggle Switch (v5)	• Required: No • Custom Entries: No	• None Set
Use Custom Read 1 Primer	Check Box(v4) Toggle Switch (v5)	• Required: No • Custom Entries: No	• None Set
Use Custom Read 2 Primer	Check Box(v4) Toggle Switch (v5)	• Required: No • Custom Entries: No	• None Set
Use Custom Recipe	Check Box(v4) Toggle Switch (v5)	• Required: Yes • Read Only: No	• Default: No
Workflow	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	• GenerateFASTQ
Workflow Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• No Index • Single Index • Dual Index • Custom

Global Custom Fields

The following table lists the global custom fields that are configured to display on the Dilute and Denature (NovaSeq 6000 v3.1) step.

Table 184 Global Custom Fields

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Flowcell Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• SP • S1 • S2 • S4
Loading Workflow Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User Interaction

1. On the Placement screen, do the following actions:
   - Drag the pool into the library tube in the Placed Samples area.
   - Scan or type the barcode of the library tube into the Library Tube field.
2. Select Record Details. After exiting the Placement screen, the Check Library Tube Barcode automation checks that the library tube barcode conforms to the barcode mask [A-Za-z]{2}[0–9]{7}-[A-Za-z]{3}. If not, an error message displays.
3. On the Record Details screen, the Reagent Lot Tracking section allows tracking of the NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. Select from the active lots displayed in each drop-down list.

   To make reagent lots available for selection in the Reagent Lot Tracking section, configure and activate them on the Reagents and Controls screen (Clarity LIMS v4) or the Configuration > Consumables screen (Clarity LIMS v5).

   - The fields that display on the Record Details screen are used to set up the run and generate the sample sheet file. Some of these fields are autopopulated and some must be completed manually. See the following table for details.

Table 185 Fields That Display on Record Details Screen of Dilute and Denature (NovaSeq 6000 v3.1) Step

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.

Field	Value
BaseSpace Sequence Hub Configuration	Select from preset options: Not Used, Run Monitoring Only, Run Monitoring and Storage.
Workflow	Automatically populated with preset, GenerateFASTQ.
Workflow Type	Select from preset options: No Index, Single Index, Dual Index, Custom.
Index Read 1	Select from preset options: 0, 6, 8 - or type a value between 0 and 20.
Index Read 2	Select from preset options: 0, 6, 8 - or type a value between 0 and 20.
Paired End	Select from preset options: True or False.
Read 1 Cycles	Select from preset options: 251, 151, 101, 51 - or type a value between 1 and 251. Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Read 2 Cycles	Select from preset options: 251, 151, 101, 51 - or type a value between 1 and 251. Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Use Custom Read 1 Primer	Select if applicable.
Use Custom Read 2 Primer	Select if applicable.
Use Custom Index Read 1 Primer	Select if applicable.
Reverse Complement Workflow	Select Yes (by default) for v1.5 reagents in NovaSeq Control Software v1.7 and onwards. Select No for v1 reagents.
Use Custom Recipe	Select if applicable.
Custom Recipe Path	If the Use Custom Recipe option is selected, enter the path to the custom recipe file to be used.
UMI—Read 1 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 1. Leave blank if not applicable.
UMI—Read 2 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 2. Leave blank if not applicable.

Field	Value
UMI—Read 1 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.
UMI—Read 2 Start From Cycle	Enter the cycle number that Read 2 starts from. Leave blank if not applicable.
Output Folder	Enter the network path for sequencing run folder. For example: \\networkshare\run_data

4. On the Record Details screen, select Validate Run Setup and Generate Sample Sheet.
   This selection triggers the automation script, which completes the following actions:
   - Validates the information provided to set up the run.
   - Generates the sample sheet file and attaches it to the placeholder in the Files area of the Record Details screen.
   - Sets the value of the next step to Remove from workflow. The Routing Script automation expects this value and requires it to successfully advance samples to the next step.
5. Select Next Steps.
   On the Assign Next Steps screen, the Next Step field for all samples is populated with Remove from workflow.

   Do not change this value. If Next Step is not set to Remove from workflow, the routing script does not route samples correctly.
6. Select Finish Step.

On exit from the step:

- The Routing Script automation is triggered and samples are routed to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.1 protocol.
- In Lab View, the pool of samples is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.) step.

At this point in the workflow, the user interaction ends. The integration automatically starts the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.1) step and data from the run is parsed back into the Clarity LIMS.

Protocol 3: NovaSeq Xp (NovaSeq 6000 v3.1)

Samples are routed to this protocol if their Loading Workflow Type value is set to NovaSeq Xp.

Samples are pooled and added to lanes on the NovaSeq flow cell type selected in the Define Run Format (NovaSeq 6000 v3.1) step. At the end of this protocol, the flow cell is sent to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.1) protocol.

This protocol contains the following three steps:

- Step 1: Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.1)
- Step 2: Dilute, Denature, and ExAmp (NovaSeq 6000 v3.1)
- Step 3: Load to Flowcell (NovaSeq 6000 v3.1)

Step 1: Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.1)

Manually place libraries into a pool.

Create only one pool per step.

Step input: NTP (normalized libraries)

Step output: Bulk pool

The following automations are configured on the step:

- Calculate Volumes
- Set Next Step
- Validate Inputs Flowcell Type and Single Pool
- Validate Unique Indexes (Not used. This functionality is handled by the core Clarity LIMS configuration for pooling.)

These automations are described in the following section, in the order in which they are triggered at run time.

Validate Inputs Flowcell Type and Single Pool

Automatically triggered on exit of the Pooling screen, this automation completes the following actions:

- Checks that all samples in the pool have the same Flowcell Type assigned to them.
- Checks that only one pool has been created.
- Changes the value of error and logging messages to reference the type of pool passed as 'bulk' pool.

```
/opt/gls/clarity/bin/java \
-jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar \
script:validate_flowcell_for_input_pools \
-i {stepURI:v2} \
-u {username} \
-p {password} \
-l {compoundOutputFileLuid1} \
-validateSingleOutput true \
-poolType bulk"
```

Calculate Volumes

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Triggered when the Calculate Volumes button is selected on the Record Details screen, this automation completes the following actions:

- Calculates the number of samples in the pool:

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: + 1
```

- Sets the value of the Bulk Pool Volume (µl) and PhiX Volume (µl) field, based on the selected Flowcell Type:

```
step.::PhiX Volume (µl):: = !step.hasValue(::% PhiX (0.25nM) Spike-In::) ? 0 :
step.::% PhiX (0.25nM) Spike-In::;
if (input.::Flowcell Type:: == ::SP::) {
step.::Bulk Pool Volume (µl):: = step.'Number of Lanes to Sequence' * 18 + 30;
step.::PhiX Volume (µl):: *= 1.9;
};
if (input.::Flowcell Type:: == ::S1::) {
step.::Bulk Pool Volume (µl):: = step.'Number of Lanes to Sequence' * 18 + 30;
step.::PhiX Volume (µl):: *= 1.9;
};
if (input.::Flowcell Type:: == ::S2::) {
step.::Bulk Pool Volume (µl):: = step.'Number of Lanes to Sequence' * 22 + 30;
step.::PhiX Volume (µl):: *= 1.9;
};
if (input.::Flowcell Type:: == ::S4::) {
step.::Bulk Pool Volume (µl):: = step.'Number of Lanes to Sequence' * 30 + 30;
step.::PhiX Volume (µl):: *= 2.2;
};
if (step.::PhiX Volume (µl):: == 0) {
step.::PhiX Volume (µl):: = ::::;
};
```

- Calculates the Per Sample Volume (µl) to be added to the pool:

```
input.::Per Sample Volume (µl)::= (((input.::Final Loading Concentration (pM)::*
5/ 1000) / input.::Normalized Molarity (nM)::) * step.::Bulk Pool Volume (µl)::)
/ step.::Number of Samples in Pool::
```

To ensure accurate pipetting of each sample in a pool for sequencing, the Per Sample Volume (µl) value must be equal to or higher than the Minimum Per Sample Volume (µl) (default value set at 5, may be edited).

Assuming the default Minimum Per Sample Volume (µl) value of 5, completes the following steps for a given batch:

1. If the smallest Per Sample Volume (µl) value is less than 5, automatically assigns a value of 5 to the sample Adjusted Per Sample Volume (µl) field.
2. Adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

- Calculates the Total Sample Volume (µl) field value:

```
step.::Total Sample Volume (µl):: = step.::Total Sample Volume (µl):: +
input.::Adjusted Per Sample Volume (µl)::
```

- If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) field value:

```
if (step.::Total Sample Volume (µl):: >= step.::Bulk Pool Volume (µl)::)
{output.::RSB Volume (µl):: = 0} else {output.::RSB Volume (µl):: = step.::Bulk
Pool Volume (µl):: - step.::Total Sample Volume (µl)::} ;
```

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs:

```
output.::Flowcell Type::= input.::Flowcell Type::;output.::Loading Workflow
Type::= input.::Loading Workflow Type::;
```

- Uses the NovaSeq_Xp_Bulk_Pool.csv and NovaSeq_Xp_Bulk_Pool2.csv template files to generate a single CSV file containing information about the bulk pool and the samples it contains. The generated file is available for download on the Step Setup screen of the following step - Dilute, Denature, and ExAmp (NovaSeq 6000 v3.1).

```
&& /opt/gls/clarity/bin/java \-jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar \
  -i {stepURI:v2} \
  -u {username} \
  -p {password} \
  -l {compoundOutputFileLuid1} \ script:driver_file_generator \
  -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_Bulk_
Pool.csv \
  -o {compoundOutputFileLuid0}.csv \ script:driver_file_generator \
  -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_Bulk_
Pool2.csv \
  -o append.csv \
&& cat append.csv >> {compoundOutputFileLuid0}.csv"
```

Set Next Step

Automatically triggered on exit of the Record Details screen, this automation completes the following actions:

- Copies the Flowcell Type values from the step inputs to the step outputs.
- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ; nextStep = ::ADVANCE::
```

Step UDFs/Custom Fields

There are seven fields defined on the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.1) step.

The following table lists Clarity LIMS v4 and Clarity LIMS v5 field configuration details.

Table 186 Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.1) Step Custom Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Bulk Pool Volume (µl) (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2
Number of Lanes to Sequence	Numeric	• Required: Yes • Read Only: No	• Decimal Places Displayed: 0
Total Sample Volume (µl) (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Default: 0 • Decimal Places Displayed: 0
Minimum Per Sample Volume (µl)	Numeric	• Required: Yes • Read Only: No	• Default: 5 • Decimal Places Displayed: 2
Number of Samples in Pool (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Default: 0 • Decimal Places Displayed: 0

Global Custom Fields

The following table lists the global custom fields that are configured to display on the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.1) step.

Table 187 Global Custom Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Configured on Analyte in Clarity LIMS v4 and Derived Sample in Clarity LIMS v5:			
RSB Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Flowcell Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• SP • S1 • S2 • S4
Loading Workflow Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User Interaction

1. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucket, and select View Ice Bucket.
2. On the Ice Bucket screen, select Begin Work.
3. On the Pooling screen:
   - Drag samples over into the Pool Creator to create a pool.
   - Name the pool or accept the default name (Pool #1).
   - Select Record Details.

   On exit from the Pooling screen, the Validate Inputs Flowcell Type and Single Pool automation is triggered. The automation checks that all samples in the pool are assigned the same Flowcell Type and only one pool has been created.
4. On the Record Details screen in the Step Details area, complete the two required fields and one optional field, as needed:
   - Number of Lanes to Sequence—The value entered in this field is used in volume calculations, to make sure that volumes are sufficient for the number of times the pool is sequenced.

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- Minimum Per Sample Volume (µl)—In many applications, and particularly where robotics are used, a minimum sample volume is required. The value in this field is used to calculate how much of each sample is included in the pool.
- % PhiX (0.25nM) Spike-In—The value in this field is used to calculate the volume of PhiX v3 control to be included in the pool for the given percentage of spike-in. The field is optional and maybe edited if necessary.

- The field is populated with the configured default value, 5 µl, but may be edited if necessary.

5. In the Sample Details table, select the pool icon to view details on the pool composition.
6. Select Calculate Volumes. This selection triggers the Calculate Volumes automation, which completes volume calculations based on the selected Flowcell Type:
   - Calculates Bulk Pool Volume (µl) value.
     - Calculates the Per Sample Volume (µl) value to be added to the pool.
     - Calculates the Total Sample Volume (µl) value.
     - If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) value.
     - Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
     - Generates the Calculation File (CSV) and attaches it to the step. This file contains volume information about the samples and RSB buffer to add to the pool. Select the file to download it, then open it in Excel.
7. Select Next Steps. This selection triggers the Set Next Step automation. This automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol, Dilute, Denature, and ExAmp (NovaSeq 6000 v3.1).
   On the Assign Next Steps screen, the next step is already set to Dilute and Denature (NovaSeq 6000 v3.1).
8. Select Finish Step.
   At the end of this step, the pool of samples automatically advances to the Dilute and Denature (NovaSeq 6000 v3.1) step.

Step 2: Dilute, Denature and ExAmp (NovaSeq 6000 v3.1)

In this step, pooled samples are denatured and diluted by the addition of DPX, NaOH, Tris-HCl, and RSB. Manually create working pools based on the number of lanes you want to sequence.

Step input: Bulk pool

Step output: Working pool - variable number, choose how many working pools to create per bulk pool

The following automations are configured on the step:

- Calculate Volumes
- Set Next Step

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- Validate Inputs Flowcell Type

The automations are described in the following section, in the order in which they are triggered at run time.

Validate Inputs Flowcell Type

Automatically triggered at the beginning of the step, this automation invokes the validate_flowcell_for_input_pools script. This script completes the following actions:

- Checks the inputs to the step and validates that the Flowcell Type field is set to a valid value (SP, S1, S2, or S4) and that all inputs have the same value for this field.
- The container type selected matches the value in the Flowcell Type field.
- Validates that the number of outputs matches the number of lanes on the selected flow cell type. If validation fails, an error message informs that the number of working pools does not match the number of lanes available on the flow cell.

```
bash -l -c "/opt/gls/clarity/bin/java \
-jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar \
script:validate_flowcell_for_input_pools \
-i {stepURI:v2} \
-u {username} \
-p {password} \
-l {compoundOutputFileLuid1}"
```

Calculate Volumes

Automatically triggered on entry to of the Record Details screen, this automation sets the value of the following fields based on the Flowcell Type:

- BP Aliquot Volume (µl)
- NaOH Volume (µl)
- Tris-HCl Volume (µl)
- DPX1 Volume (µl)
- DPX2 Volume (µl)
- DPX3 Volume (µl)
- Mastermix per Lane (µl)

```
if ( input.::Flowcell Type:: == ::SP:: ) {
output.::BP Aliquot Volume (µl):: = 18 ;
output.::NaOH Volume (µl):: = 4.50 ;
output.::Tris-HCl Volume (µl):: = 4.50 ;
step.::DPX1 Volume (µl):: = 126 ;
```

```
step.::DPX2 Volume (µl):: = 18 ;
step.::DPX3 Volume (µl):: = 66 ;
output.::Mastermix per Lane (µl):: = 63
};

if (input.::Flowcell Type:: == ::S1::) {
output.::BP Aliquot Volume (µl):: = 18;
output.::NaOH Volume (µl):: = 4.50;
output.::Tris-HCl Volume (µl):: = 4.50;
step.::DPX1 Volume (µl):: = 126;
step.::DPX2 Volume (µl):: = 18;
step.::DPX3 Volume (µl):: = 66;
output.::Mastermix per Lane (µl):: = 63
};
if (input.::Flowcell Type:: == ::S2::) {
output.::BP Aliquot Volume (µl):: = 22;
output.::NaOH Volume (µl):: = 5.50;
output.::Tris-HCl Volume (µl):: = 5.50;
step.::DPX1 Volume (µl):: = 126;
step.::DPX2 Volume (µl):: = 18;
step.::DPX3 Volume (µl):: = 66;
output.::Mastermix per Lane (µl):: = 77
};
if (input.::Flowcell Type:: == ::S4::) {
output.::BP Aliquot Volume (µl):: = 30;
output.::NaOH Volume (µl):: = 7.50;
output.::Tris-HCl Volume (µl):: = 7.50;
step.::DPX1 Volume (µl):: = 315;
step.::DPX2 Volume (µl):: = 45;
step.::DPX3 Volume (µl):: = 165;
output.::Mastermix per Lane (µl):: = 105
};
```

The automation also completes the following actions:

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs:

```
output.::Flowcell Type::= input.::Flowcell Type::;
output.::Loading Workflow Type::= input.::Loading Workflow Type::;
```

- Uses the NovaSeq_Xp_Working_Pool.csv and NovaSeq_Xp_Working_Pool2.csv template files to generate a single CSV file containing information about the DPX volume and the volume of BP Aliquot, Mastermix, NaOH, and Tris-HCl to add per working pool. The generated file is available for download on the Step Setup screen of the following step - Load to Flowcell (NovaSeq 6000 v3.1).

```
and /opt/gls/clarity/bin/java \
-jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/DriverFileGenerator.jar \
 -i {stepURI:v2} \
 -u {username} \
 -p {password} \
 -l {compoundOutputFileLuid1} \script:driver_file_generator \
 -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_Working_
Pool.csv \
 -o {compoundOutputFileLuid0}.csv \script:driver_file_generator \
 -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_Working_
Pool2.csv \
 -o append.csv \
&& cat append.csv >> {compoundOutputFileLuid0}.csv"
```

Set Next Step

Automatically triggered on exit of the Record Details screen, this automation:

- Copies the Flowcell Type values from the step inputs to the step outputs.
- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ; nextStep = ::ADVANCE::
```

Step UDFs/Custom Fields

There are three fields defined on the Dilute, Denature, and ExAmp (NovaSeq 6000 v3.1) step. These field values are set by a script and are not editable while running the step.

The following table lists field configuration details.

Table 188 Dilute, Denature, and ExAmp (NovaSeq v3.1) Step Custom Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
DPX1 Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
DPX2 Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0
DPX3 Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0

Global Custom Fields

The following table lists the global custom fields that are configured to display on the Dilute, Denature, and ExAmp (NovaSeq 6000 v3.1) step.

Table 189 Global Custom Fields

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Configured on Analyte in Clarity LIMS v4 and Derived Sample in Clarity LIMS v5:			
BP Aliquot Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0
Flowcell Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• SP • S1 • S2 • S4
Loading Workflow Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
Mastermix per Lane (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0
NaOH Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Tris-HCl Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2

User Interaction

1. On the Queue screen, add the pool to the Ice Bucket and select View Ice Bucket.
2. On the Ice Bucket screen, (optionally) set the number of derivatives to create (they are placed into the flow cell lanes) and select Begin Work.
   On entry to the Record Details screen, the Calculate Volumes automation is triggered. This automation sets the following values based on the selected Flowcell Type (calculation details are provided in the previous Calculate Volumes automation section):
   - BP Aliquot Volume (µl)
   - NaOH Volume (µl)
   - Tris-HCl Volume (µl)
   - DPX1 Volume (µl)
   - DPX2 Volume (µl)
   - DPX3 Volume (µl)
   - Mastermix per Lane (µl)
   - Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
   - Generates the Calculation File (CSV) and attaches it to the step. This file contains information about the DPX Mastermix volume, and the volume of Mastermix, NaOH, and Tris-HCl to add per working pool. Select the file to download it and then open it in Excel.
3. On the Record Details screen, the Reagent Lot Tracking section tracks the DPX1, DPX2, DPX3, NaOH, RSB, and Tris-HCl reagent lots. Select from the active lots displayed in each drop-down list.

   > To make reagent lots available for selection in the Reagent Lot Tracking section, first configure and activate them. Do this action on the Reagents and Controls screen (Clarity LIMS v4x) or the Configuration > Consumables screen (Clarity LIMS v5x).

   - In the Step Details area, the DPX1, DPX2, and DPX3 reagent volume values are populated. (These values are set by a script and are not editable while running this step.)
   - In the Sample Details table the following actions are available:
     - Select the pool icon to view details on the working pool composition.
     - The BP Aliquot, Mastermix per lane, NaOH, and Tris-HCl volume values for each working pool are populated. (These values are set by a script and are not editable while running the step.)
     - The working pool number is appended to the bulk pool name. This feature quickly identifies which working pools are derived from the same bulk pool.
   - In the Files area, select the Calculation File (CSV) to download it and then open it in Excel. View information about the DPX Mastermix volume, and the volume of Mastermix, NaOH. and Tris-HCl to add per working pool.
   - Select Next Steps to trigger the Set Next Step automation.

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4. On the Assign Next Steps screen, the next step is set to Load to Flowcell (NovaSeq 6000 v3.0). Select Finish Step.

Step 3: Load to Flowcell (NovaSeq 6000 v3.1)

In this step, scan the flow cell barcode into the Clarity LIMS and manually place the working pools into the lanes of the flow cell for the NovaSeq run. This step validates the run setup information and generates the sample sheet file.

Step input: Working pool

Step output: Flow cell (output containers: SP, S1, and S2 with 2 lanes, and S4 with 4 lanes)

The following items are configured on the step:

- Validate Run Setup and Generate Sample Sheet
- Validate Flowcell Barcode
- Validate Inputs and Selected Container

The automations are described in the following section, in the order in which they are triggered at run time.

Validate Inputs and Selected Container

Automatically triggered at the beginning of the step, this automation invokes the validate_flowcell_for_input_pools and validateSelectedContainer scripts. These scripts validate the step inputs and the container selected by the user, as follows.

- Checks that the Flowcell Type field has been set to a valid value (SP, S1, S2, or S4) and that all inputs have the same value.
- Checks that the number of outputs matches the number of lanes on the selected flow cell type. If validation fails, an error message informs that the number of working pools does not match the number of lanes available on the flow cell.
  The following table defines the number of samples allowed:

Flow Cell Type	Number of Working Pools
SP	2
S1	2
S2	2
S4	4

- Checks that the container type selected on entry to the Placement screen matches the value in the Flowcell Type field. If validation fails, an error message displays.

```
bash -l -c "/opt/gls/clarity/bin/java \
```


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```
-jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar
\script:validate_flowcell_for_input_pools \
  -i {stepURI:v2} \
  -u {username} \
  -p {password} \
  -l {compoundOutputFileLuid1} \
  -validateSelectedContainer true"
```

Validate Flowcell Barcode

Automatically triggered on exit of the Placement screen, this automation validates the flow cell barcode scanned into the Clarity LIMS by the user, using the following logic:

```
if (input.::Flowcell Type:: == ::SP:: && !output.container.name.matches( ::[A-
Za-z0-9]{5}DRXX:: ) ) { \
  fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: ) \
} ; \
if (input.::Flowcell Type:: == ::S1:: && !output.container.name.matches( ::[A-
Za-z0-9]{5}DRXX:: ) ) { \
  fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: ) \
} ; \
if (input.::Flowcell Type:: == ::S2:: && !output.container.name.matches( ::[A-
Za-z0-9]{5}DMXX:: ) ) { \
  fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: ) \
} ; \
if (input.::Flowcell Type:: == ::S4:: && !output.container.name.matches( ::[A-
Za-z0-9]{5}DSXX:: ) ) { \
  fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: ) \
} ;
```

- Copies the Flowcell Type and Loading Workflow Type field values from step inputs to outputs:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ; \
output.::Loading Workflow Type:: = input.::Loading Workflow Type::'
```

Validate Run Setup and Generate Sample Sheet

Automatically triggered by selecting a button on the Record Details screen, this automation copies the Flowcell Type from the step input to the Run Mode field (hidden):

```
step.::Run Mode:: = input.::Flowcell Type::
```

- Checks the Flowcell field value.
  - If Flowcell Type value is not SP, checks that the value of both Read 1 Cycles and Read 2 Cycles is 151 or less. If value is greater than 151, displays an error message.

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```
if (input.::Flowcell Type::!=::SP:: && step.::Read 1 Cycles:: > 151) {
fail(::Read 1 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
}
if (input.::Flowcell Type::!=::SP:: && step.::Read 2 Cycles:: > 151) {
fail(::Read 2 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
}
```

- Validates the parameters entered on the Record Details screen, which are used to set up the run and generate the sample sheet file. See *Respond to Validation/Recipe Request Call from Instrument* section for more details.
  - Experiment Name may only contain alphanumeric, dash, or underscore characters. Spaces are not permitted.
  - When Workflow Type = No Index, both Index Read 1 and Index Read 2 must be zero.
  - When Workflow Type = Single Index, Index Read 1 must be greater than zero and Index Read 2 must be zero.
  - When Workflow Type = Dual Index, both Index Read 1 and Index Read 2 must be greater than zero.

```
if(!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) {
fail(::Experiment Name contains prohibited characters. Allowed characters are:
a-z, A-Z, 0-9, -, and _::)
};
if (step.::Workflow Type:: == ::No Index::) {
if (step.::Index Read 1:: != 0 || step.::Index Read 2:: != 0) {
fail(::Index Read 1 and Index Read 2 must be 0 if No Index is selected.::)
}
} else if (step.::Workflow Type:: == ::Single Index::) {
if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) {
fail(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if Single
Index is selected.::)
}
} else {
if (step.::Index Read 1:: == 0 || step.::Index Read 2:: == 0) {
fail(::Index Read 1 and Index Read 2 must be greater than 0 if Dual Index is
selected.::)
}
};
```

- Checks the Paired End and Read 2 Cycles field values.
  - If Paired End = True and Read 2 Cycles value is 0, an error displays.
  - If Paired End = False, Read 2 Cycles must be zero and UMI - Read 2 Length and UMI - Read 2 Start From Cycle must be empty.

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```
if (step.::Paired End::.toBoolean()) {
if (step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) {
fail(::Read 1 Cycles and Read 2 Cycles must be greater than 0 if Paired End is
True.::)
}
} else {
if (step.::Read 2 Cycles:: != 0) {
fail(::Read 2 Cycles must be 0 if Paired End is False.::)
};
if (step.hasValue(::UMI - Read 2 Length::) || step.hasValue(::UMI - Read 2 Start
From Cycle::)) {
fail(::UMI - Read 2 Length and UMI - Read 2 Start From Cycle cannot be defined
if Paired End is False.::)
}
};
```

- Validates UMI Settings:
  - If UMI - Read 1 Length has value, then UMI - Read 1 Start From Cycle must have value.
  - If UMI - Read 1 Length has no value, then UMI - Read 1 Start From Cycle must not have value.
  - If UMI - Read 2 Length has value, then UMI - Read 2 Start From Cycle must have value.
  - If UMI - Read 2 Length has no value, then UMI - Read 2 Start From Cycle must not have value.
  - If UMI - Read 1 Length has not value, then UMI - Read 2 Length must not have value.
  - If UMI - Read 1 Start From Cycle has no value, then UMI - Read 2 Start From Cycle must not have value.

```
if (step.hasValue(::UMI - Read 1 Length::) && !step.hasValue(::UMI - Read 1
Start From Cycle::)) {
fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 1
Length is greater than 0.::)
};
if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 1
Start From Cycle::)) {
fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 1 Start From
Cycle is greater than 0.::)
};
if (step.hasValue(::UMI - Read 2 Length::) && !step.hasValue(::UMI - Read 2
Start From Cycle::)) {
fail(::UMI - Read 2 Start From Cycle must be greater than 0 if UMI - Read 2
Length is greater than 0.::)
};
```


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```
if (!step.hasValue(::UMI - Read 2 Length::) && step.hasValue(::UMI - Read 2
Start From Cycle::)) {
fail(::UMI - Read 2 Length must be greater than 0 if UMI - Read 2 Start From
Cycle is greater than 0.::)
};
if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 2
Length::)) {
fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 2 Length is
greater than 0.::)
};
if (!step.hasValue(::UMI - Read 1 Start From Cycle::) && step.hasValue(::UMI -
Read 2 Start From Cycle::)) {
fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 2
Start From Cycle is greater than 0.::)
};
```

- Outputs the Settings Header in sample sheet if UMI - Read 1 Length or UMI - Read 2 Length have value.

```
step.::Settings Header:: = step.hasValue(::UMI - Read 1 Length::) ||
step.hasValue(::UMI - Read 2 Length::) ? ::[Settings]:: : ::::;
```

- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol - AUTOMATED - Run (NovaSeq 6000) v3.0.

```
nextStep = ::ADVANCE::
```

- Generates the sample sheet and attaches it to the step. (See Sample Sheet Contents for more information.) The sample sheet template depends on the selected value for Reverse Complete Workflow.

```
&& if [ '{udf:Reverse Complement Workflow}' == 'true' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Reverse_Complement_Samplesheet.csv'; \
elif [ '{udf:Reverse Complement Workflow}' == 'false' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Samplesheet.csv'; \
else echo 'UDF Reverse Complement Workflow is empty or undefined.'; exit -1; fi
\
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/$templateFilename \
-o {compoundOutputFileLuid0}.csv \
-q true \
-destLIMSID {compoundOutputFileLuid0} \
```

```
-l {compoundOutputFileLuid1}"
```

Step UDFs/Custom Fields

There are 23 fields defined on the Load to Flowcell (NovaSeq 6000 v3.1) step.

The following table lists the field configuration details.

Table 190 Load to Flowcell (NovaSeq 6000 v3.1) Step Custom Field Configuration

Field Name	Field Type	Field Contraints/Options	Preset Values/Additional Options and Dropdown Items
BaseSpace Sequence Hub Configuration	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• Not Used • Run Monitoring Only • Run Monitoring and Storage
Custom Recipe Path	Single-line Text (v4) Text (v5)	• Required: No • Read Only: No	Not applicable
Experiment Name	Single-line Text (v4) Text (v5)	• Required: Yes • Read Only: No	Not applicable
Index Read 1	Numeric (v4) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 0 • 6 • 8 • Range From: 0 to 20 • Decimal Places Displayed: 0
Index Read 2	Numeric (v4) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 0 • 6 • 8 • Range From: 0 to 20 • Decimal Places Displayed: 0
Library Tube Barcode	Single-line Text (v4) Text (v5)	• Required: Yes • Read Only: No	Not applicable

Field Name	Field Type	Field Contraints/Options	Preset Values/Additional Options and Dropdown Items
Paired Ends	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• True • False
Read 1 Cycles	Numeric (v4) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 251* • 151 • 101 • 51 • Range From: 1 to 251 • Decimal Places Displayed: 0 *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151
Read 2 Cycles	Numeric (v4) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 251* • 151 • 101 • 51 • Range From: 1 to 251 • Decimal Places Displayed: 0 *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151
Reverse Complement Workflow	Check Box (v4) Toggle Switch (v5)	• Required: Yes • Read Only: No	• Default: Yes
Run Mode (not displayed in user interface)	Single-line Text (v4) Text Dropdown (v5)	• Required: No • Read Only: Yes • Custom Entries: No	• SP • S1 • S2 • S4

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Field Name	Field Type	Field Contraints/Options	Preset Values/Additional Options and Dropdown Items
Setting Header (not displayed in user interface)	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable
UMI—Read 1 Length	Numeric (v4) Numeric (v5)	• Required: No • Read Only: Yes	Not applicable
UMI—Read 1 Start From Cycle	Numeric (v4) Numeric (v5)	• Required: No • Read Only: Yes	Not applicable
UMI—Read 2 Length	Numeric (v4) Numeric (v5)	• Required: No • Read Only: Yes	Not applicable
UMI—Read 2 Start From Cycle	Numeric (v4) Numeric (v5)	• Required: No • Read Only: Yes	Not applicable
Use Custom Index Read 1 Primer	Check Box (v4) Toggle Switch (v5)	• Required: No • Read Only: No	• None Set
Use Custom Read 1 Primer	Check Box (v4) Toggle Switch (v5)	• Required: No • Read Only: No	• None Set
Use Custom Read 2 Primer	Check Box (v4) Toggle Switch (v5)	• Required: No • Read Only: No	• None Set
Use Custom Recipe	Check Box (v4) Toggle Switch (v5)	• Required: Yes • Read Only: No	• Default: No
Workflow	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	• GenerateFASTQ
Workflow Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• No Index • Single Index • Dual Index • Custom

Global Custom Fields

The following table lists the global custom fields that are configured to display on the Load to Flowcell (NovaSeq 6000 v3.1) step.

Table 191 Global Custom Fields Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Configured on Analyte in Clarity LIMS v4 and Derived Sample in Clarity LIMS v5:			
Flowcell Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• SP • S1 • S2 • S4
Loading Workflow Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User Interaction

1. On the Ice Bucket screen, complete the following actions:
   - Select the appropriate flow cell type from the Destination Container drop-down list in the Container Options panel.
   - Select Begin Work.
2. On the Placement screen, complete the following actions:
   - Drag one or more of the pools from the left of the screen into the Placed Samples area to the right.
   - Scan or type the barcode of the flow cell into the flow cell field.
   - Select Record Details.

   On exit of the Placement screen, the Validate Flowcell Barcode automation validates the container barcode (described previously).
3. Set up the run and generate the sample sheet file using the fields that display on the Record Details screen. Some of these fields are autopopulated and some must be completed manually.
   The following table lists the fields that display on Record Details screen of Load to Flowcell (NovaSeq 6000 v3.1) step.

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Table 192 Fields That Display on Record Details Screen of Load to Flowcell (NovaSeq 6000 v3.1) Step

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.
BaseSpace Sequence Hub Configuration	Select from preset options: Not Used, Run Monitoring Only, Run Monitoring and Storage.
Workflow	Automatically populated with preset - GenerateFASTQ.
Workflow Type	Select from preset options: No Index, Single Index, Dual Index, Custom.
Index Read 1	Select from preset options: 0, 6, 8 - or type a value between 0 and 20.
Index Read 2	Select from preset options: 0, 6, 8 - or type a value between 0 and 20.
Paired End	Select from preset options: True or False.
Read 1 Cycles	Select from preset options: 251, 151, 101, 51 - or type a value between 1 and 251. Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Read 2 Cycles	Select from preset options: 251, 151, 101, 51 - or type a value between 1 and 251. Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Use Custom Read 1 Primer	Select if applicable.
Use Custom Read 2 Primer	Select if applicable.
Use Custom Index Read 1 Primer	Select if applicable.
Reverse Complement Workflow	Select Yes (by default) for v1.5 reagents in NovaSeq Control Software v1.7 and onwards. Select No for v1 reagents.
Use Custom Recipe	Select if applicable.
Custom Recipe Path	If Use Custom Recipe selected, enter the path to the custom recipe file to be used.
UMI - Read 1 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 1. Leave blank if not applicable.

Field	Value
UMI - Read 2 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 2. Leave blank if not applicable.
UMI - Read 1 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.
UMI - Read 2 Start From Cycle	Enter the cycle number that Read 2 starts from. Leave blank if not applicable.
Output Folder	Enter the network path for sequencing run folder. For example: \\networkshare\run_data
Library Tube Barcode	Scan the library tube barcode.

4. On the Record Details screen, select Validate Run Setup and Generate Sample Sheet.
   This selection triggers the automation script, which completes the following actions:
   - Validates the information provided to set up the run.
   - Generates the sample sheet file and attaches it to the placeholder in the Files area of the Record Details screen.
5. Select Next Steps.
   On the Assign Next Steps screen, the Next Step field for all samples is populated with Mark protocol as complete.
6. Select Finish Step.
   In Lab View, the pooled samples are queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.1) step.

At this point in the workflow, the user interaction ends. The integration automatically starts the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.1) step and data from the run is parsed back into the Clarity LIMS.

Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.1)

This final protocol contains one fully automated step, AUTOMATED- NovaSeq Run (NovaSeq 6000 v3.1), described in the following section.



This is a fully automated step. Do not add samples to the Ice Bucket or start the step manually.

Step 1: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.1)

Step input: Library tube from NovaSeq Standard or flow cell from NovaSeq Xp protocol

Step output: Result file/measurement



Do not start this step manually. It is a fully automated step and the sequencing service does not update samples correctly if they have been manually started.

In this step, pooled samples are sequenced on the NovaSeq instrument and the run metrics are recorded in Clarity LIMS.

The Automated - NovaSeq Run (NovaSeq 600 v3.1) step contains two automations:

- Set Next Steps
- Update Lane Number

By default, the Set Next Steps automation is disabled (set to Not Used) and the Sequencer API sets the next step for samples.

Set Next Steps

If necessary, override the default next step behavior used by the Sequencer API by performing the following steps:

1. Enable the automation and use it to set custom next step behavior and generate a log file. By default, the automation sets the next step to ADVANCE and the samples complete the protocol.
2. Configure the automation to trigger automatically on exit of the Record Details screen.

Update Lane Number

This automation triggers automatically upon entry to the Record Details screen.

Do not disable or modify this automation to ensure the Lane Number displays properly.

Step UDFs/Custom Fields

The following table lists the run parameters parsed from the RunParameter.xml file and mapped to fields configured on the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.1) step.

Table 193 Run Parameters - Field Mappings

Step UDF/ Custom Field Name
Current Cycle
Current Read
Firmware Version
Flow Cell Expiration Date
Flow Cell ID
Flow Cell Lot Number
Flow Cell Mode
Flow Cell Part Number

Step UDF/ Custom Field Name
Flow Cell Side
Instrument Control Software Version
Instrument ID
Instrument Type
Lane Counter
Loading Workflow Type
Output Folder
RTA Version
Run ID
Run Status
Sequencing Log

Global Custom Fields

The following global custom fields are used to capture the run metrics in Clarity LIMS.

- % Aligned R1
- % Aligned R2
- % Bases >=Q30 R1
- % Bases >=Q30 R2
- % Error Rate R1
- % Error Rate R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- %PF R1
- %PF R2
- Cluster Density (K/mm^2) R1
- Cluster Density (K/mm^2) R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- Reads PF (M) R1

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- Reads PF (M) R2
- Yield PF (Gb) R1
- Yield PF (Gb) R2

Note the following details:

- Values are aggregated across all lanes. Some values, for example Yield PF (Gb) R1, are summed while others are averaged.
- The names listed previously are the default global custom field names installed with the NovaSeq Integration v3.3.0 configuration provided in the Preconfigured Workflow Package (PCWP) v2.0.1 or later.
- All global configuration fields are configured on the Container entity.
- All field names are configurable via the Clarity LIMS Configuration > Custom Fields screen (Global Fields tab).
- If you change the field names in Clarity LIMS, also change them through the run.metricUdfNames properties file in the application.yml file for the Sequencer API (see *application.yml Properties on page 688*). All fields are configured to be visible on the Record Details screen for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.1) step (in the Sample Details table).

These global custom fields are configured on the Container entity, visible on each input, but they should not be confused with per-lane metrics.

All run metrics tracked in Clarity LIMS are overall metrics for the run. There are no per-lane metrics provided by NovaSeq Control Software currently.

How the Sequencer API Integration Works

Sample Sheet Generation

The sample sheet is generated on the step before the run, which is Dilute and Denature (NovaSeq 6000 v3.1) in the NovaSeq Standard protocol or Load to Flowcell (NovaSeq 6000 v3.1) in the NovaSeq Xp protocol. This step places samples on the library tube or flow cell that are loaded in the NovaSeq instrument.

In the default configuration, the *Validate Run Setup and Generate Sample Sheet on page 669* automation generates one CSV format sample sheet file for use with bcl2fastq v2.20 downstream analysis.

How it Works

The sample sheet is uploaded to the NVCS via the /Illumina/Sequencer/v2/sequencing-run/files endpoint to the Sequencer API. The file endpoint allows for a file to be downloaded from the Clarity LIMS using OAuth (instead of Basic Authentication), which is required for NovaSeq.

The run recipe response sets the sample sheet URL to the link to download the file from this endpoint and sets the sampleSheetRequiresOAuth value to true.

Sample Sheet Contents

The sample sheet content is controlled by the following step UDFs/custom fields that display in the Step Details area of the Record Details screen.

- Experiment Name
- Read 1 Cycles
- Read 2 Cycles
- Workflow
- Reverse Complement Workflow

See *Illumina Instrument Sample Sheets (NGS v5.17 & later)* on page 2542 for detailed information on the following items:

- Sample sheet generation script parameters and usage
- Sample sheet data and configuration options
- Enabling unique FASTQ file names per sequencing run

NovaSeq Run

The following steps outline the sequence of events that occurs when a flow cell is loaded onto the NovaSeq instrument.

User Authentication and Login

User authentication and login to the NovaSeq instrument is achieved via the getSequencerLoginURL endpoint (found at /Illumina/Sequencer/v2/sequencing-run/login). This endpoint returns the URL that the instrument uses to get an access token.

1. The Sequencer API service provides the NovaSeq Control Software (NVCS) with a URL for the Clarity LIMS login screen and the NVCS displays this screen to the user. Log in to automatically authorize the NVCS to use your account to access the Clarity LIMS.
2. Enter credentials:
   - Username and password are passed to the Clarity LIMS server to make sure that the access is valid.
   - By default, the Sequencer API service tries to contact the Clarity LIMS at http://localhost:9080/clarity/. This URL is set by the installation script, but it can be configured with the clarity.url property in the application.yml file (see *application.yml Properties on page 688*).
3. User is redirected to the Sequencer login page at /Illumina/Sequencer/v2/sequencer_login.

- If authentication is successful, the screen displays a success message. The Clarity LIMS server provides the OAuth token and username by returning them in redirect to /Illumina/Sequencer/v2/sequencer_login/Success. This redirect does not reload the page, but allows the NovaSeq to continue with run setup.
- If authentication fails, the user is left on the login screen and a warning message informs them of the failure. A background redirect to /Illumina/Sequencer/v2/sequencer_login/Error informs the NovaSeq of the failure.

Respond to Validation/Recipe Request Call from Instrument

1. The sequencing-run/recipe/novaseq endpoint consumes a POST request with a LibraryContainerI, FlowCellId, and reagents information.
   - LibraryContainerId, FlowCellId, and reagent information is provided by the NovaSeq. LibraryContainerId is required.
   - LibraryContainerId and FlowCellId should match either the library tube ID or flow cell ID of the corresponding containers in Clarity LIMS.
   - If there are no containers or multiple containers, an error is generated.
2. All populated containers whose name matches either the LibraryContainerId or FlowCellId are validated as follows.
   - All samples in the container must be queued for a sequencing run step.
   - All samples must be queued for only one sequencing run step.
   - All samples must be queued for the same sequencing run step.

   Configure the steps that are considered sequencing run steps in the novaseq.sequenceStepNames property in the application.yml file. The property is a list and each list entry must be the exact name of a sequencing step, not the name of the underlying master step/process type. See *application.yml Properties on page 688* for more details.
3. After a single container has passed validation, the run setup information (run recipe request) is returned to the NVCS via the API and the run recipe is generated.
   Note the following details:
   - The run recipe information is read from fields configured on the parent process/master step of the samples in the container. The workflow must include recipe definition in that step.
   - By default, the parent process/master step is the Dilute and Denature (NovaSeq 6000 v3.1) or Load to Flowcell (NovaSeq 6000 v3.1) step, depending on workflow.
   - The default field values match the names of the fields in the NovaSeq v3.3.0 configuration included in PCWP and IPP. The field names are configurable through the recipe.udfNames.* properties. Each of these properties corresponds to an entry in the recipe and the value of a property defines the name of the field that is used to populate that entry in the recipe.

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- It is assumed that this parent process/master step removes any illegal characters from the Experiment Name field.
- The Clarity LIMS download link to the sample sheet created on the run setup step is used for the SampleSheet value in the recipe. The name of the output of the sample sheet on the setup step can be configured through the recipe.sampleSheet.outputName property (Sample Sheet by default) in the application.yml (see *application.yml Properties on page 688* for more details). If no matching output is found, or no file was attached to the output, then the value of the recipe.sampleSheet.notAvailableValue property (Not Available by default) is used in the recipe.

4. After the recipe is generated, it is returned as a *.json file in the response, in the form defined by the Swagger definition for the API.

Run Recipe Contents

The contents of the run recipe are controlled by the sample sheet contents and the fields configured on the Dilute and Denature (NovaSeq 6000 v3.1) and Load to Flowcell (NovaSeq 6000 v3.1) steps:

Example of run recipe content is as follows:

```
{
  "run_name": "NVCS16027_SPrime_2x250",
  "run_mode": "S4",
  "workflow_type": "DualIndex",
  "librarytube_id": "NV0025867-LIB",
  "flowcell_id": "",
  "sample_loading_type": "NovaSeqStandard",
  "rehyb": false,
  "paired_end": true,
  "read1": 151,
  "read2": 151,
  "index_read1": 8,
  "index_read2": 8,
  "output_folder": "\\\\network_path\\run_data",
  "samplesheet": "<path to sample sheet on LIMS server>",
  "require_samplesheet_authentication": true,
  "usecustomrecipe": false,
  "customRecipe": null,
  "use_basespace": false,
  "basespace_mode": null,
  "use_custom_read1_primer": false,
  "use_custom_read2_primer": false,
  "use_custom_index_read1_primer": false
}
```

Respond to Sequencing Started Call from Instrument

1. Matching container for the run (library tube or flow cell) is found, and contents are validated to be queued for the correct step.

   > If no matching container is found or if the samples in the container are incorrectly queued, an error is thrown and the step is not started. This validation is also done during the run recipe request immediately prior, so this error case should not be encountered.

2. Reagent kits that are configured for the Sequencer API are checked against the configuration for the step to be run:
   - If a kit is configured for the API but does not exist in Clarity LIMS, it is created.
   - If a kit exists, but is not enabled on the step to be run, the step configuration is updated to enable it.
     - If a kit that is to be enabled is Archived, it is set to Active.
     - If a kit that is to be enabled is in Pending status, it is set to Active.
   - If any kits enabled on the step are not configured for the Sequencer API, a warning is logged and the step cannot be completed by the API without user intervention.
   - If any kits provided in the recipe request are not configured for the Sequencer API, they are ignored and a warning is logged during lot tracking.
3. The number of replicates for the step is determined by the type of flow cell being used in the run.
   - For Standard mode, this information is based on the number of lanes in the flow cell. This information is stored as part of the Sequencer API configuration.
   - For XP mode, there is always one replicate, because there is already one input tracked for each lane.
4. The automated sequencing step is started.
5. Reagent lots are attached to the step. The Flow Cell and Library Tube reagents in the request are ignored. Information from these reagents is tracked in Clarity LIMS as containers and custom fields. Any other reagents in the request are handled as follows.
   - If the reagent is not configured to be tracked, a warning is logged and the reagent is ignored.
   - Existing reagent lots are looked up by kit name (determined by Sequencer API configuration), name (the serial number in the request), and lot number (the lot number in the request).
   - If one or more matching reagent lots exist in Clarity LIMS, the newest one is used.
   - If the expiry date does not match the expiry in the request, or if the status is not set to Active, the lot is updated to have the correct expiry date and an Active status.

   Otherwise a new lot is created with the following configuration:
   - Reagent Kit—Matching kit as determined from Sequencer API configuration and step 2.
   - Name—Serial number in request.

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- Lot Number—Lot number in request.
- Expiry—Expiry date in request.
- Status—Active.

If any reagents are expected to be in the request but are not, these reagents are logged. This action prevents the step from being completed without user intervention.

6. Step UDFs/custom fields are updated with the run information parsed from the request. See *Respond to Record InterOps Metrics Call from Instrument* on page 686.
7. Any important events during this request are logged into the Sequencer API log file and in the Sequencing Log multiline global custom field on the step (assuming the step was started successfully). See *Respond to Record InterOps Metrics Call from Instrument* on page 686.

Respond to Record InterOps Metrics Call from Instrument

When the metrics endpoint receives a POST, it completes the following actions:

- Looks for a matching library tube or flow cell in Clarity LIMS.
- Find the in-progress sequencing step for that container.

When the step is found, the run metrics in the request are written to container global custom fields in Clarity LIMS.

Respond to Sequencing Completed Call from Instrument

If the sequencing run is successful, after the metrics are recorded, the API call for Sequencing Completed is received. Information is matched by Sequencing Container and the following occurs in Clarity LIMS:

1. The Current Cycle, Current Read, and Status step UDFs/custom fields are updated.
2. The multiline Sequencing Log UDF/master step field is updated.
3. A success message is recorded in the log file at the info level:

```
Sequencing run with Run ID {runId} of Step '{step.name}' ({step.limsid})
completed successfully.
```

4. The Clarity LIMS waits for any automations that are triggered automatically as it advances the step.
5. By default, the Clarity LIMS assigns the next steps on the outputs to move them on to the first configured next step. If there are no next steps, the protocol is completed.

Respond to Sequencing Errored Call from Instrument

If the sequencing run is unsuccessful, the API call for Sequencing Error is received. Information is matched by Sequencing Container and the following occurs in Clarity LIMS:

1. The Current Cycle, Current Read, and Status step UDF/custom fields are updated.
2. The multiline Sequencing Log UDF/master step field is updated.
3. A failure message is recorded in the log file at the error level:

```
Step '${Step.name}' (${Step.limsid}) failed with Run ID ${runId}.
```

4. The step is left at the Record Details screen.

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Respond to Sequencing RunEndedByUser Call from Sequencer

If the sequencing run is ended by the user, the API call for Sequencing RunEndedByUser is received. Information is matched by Sequencing Container and the following occurs in Clarity LIMS:

1. The Current Cycle, Current Read, and Status step UDFs/custom fields are updated.
2. The multiline Sequencing Log UDF/master step field is updated.
3. The following message is recorded in the log file, at the warning level:

```
"Sequencing run with Run ID ${status.runInfo.runId} of Step
'${step.configuration.name}' (${step.limsid}) aborted by user
${status.runInfo.userName}."
```

4. The step is left at the Record Details screen.

Components Installed with this Integration

The following sections describe the various components (files, properties, reagent categories / label groups, reagent kits, and containers) that are installed by default as part of this integration. Information on installed workflows, protocols, steps, and automations is provided in the previous Workflows, Protocols, and Steps section.

Illumina NovaSeq Integration v3.3.0 is distributed as an RPM package, BaseSpaceLIMS-sequencer-api. This RPM package must be installed on the Clarity LIMS server.

The BaseSpaceLIMS-sequencer-api RPM installs the following items:

- Sequencer API WAR file
- Configuration file - application.yml
- Two configuration scripts: configure_sequencer_api_proxy.sh and configure_sequencer_api_application.sh

> The NovaSeq v3 configuration is delivered through the Preconfigured Workflows Package (PCWP) v2.0.1, and Illumina Preset protocols (IPP) v2.0, which requires installation of the NGS Extensions Package v5.20.1 or later.
>
> - If the NGS Extensions Package is not already installed, or if a version earlier than v5.20.1 is installed, the latest version is installed by default with the NovaSeq integration. See Release Notes for details.
> - If NGS Extensions Package v5.20.1 or later is already installed, upgrade is not forced or required.

The following table lists the components installed by the RPM package.

Table 194 Illumina NovaSeq 6000 Integration RPM Components

Files Installed	Location and Description
Illumina#Sequencer#v2.war	/opt/gls/clarity/tomcat/current/webapps War file for NovaSeq v3.3.0 API.
configure_sequencer_api_application.sh	/opt/gls/clarity/config/ Script that configures the Sequencer API application through its external application.yml file.
configure_sequencer_api_env.sh	/opt/gls/clarity/config/ Script that configures the Clarity LIMSTomcat configuration to include Secret Util settings.
configure_sequencer_api_proxy.sh	/opt/gls/clarity/config/ Script that configures the proxy to allow communication with the Sequencer API (NovaSeq v3.3.0 API) application.
sequencer-api.conf	/etc/httpd/clarity/ Proxy setting to communicate with the Clarity LIMS.

application.yml Properties

The application.yml file is located at opt/gls/clarity/extensions/sequencing-api/.

- All properties are configured automatically during installation, but you can also configure them by editing the application.yml file and then restarting Tomcat.
- All custom field names, both for recipe and run information, are represented by properties in the file.
- For all custom fields, the names (values in this file) can be changed. However, the property names cannot be changed, removed, or added to. For example, you can change Loading Workflow Type but you cannot change recipe.udfNames.sampleLoadingType.

Table 195 application.yml Properties Installed

Property	Description
spring.profiles.active	Tells the application that it is running deployed in Tomcat. Default value: tomcat (Do not change)
clarity.url	Base URL that the Sequencer API service uses to contact the Clarity LIMS. The installation script (configure_sequencer_api_application) prompts for this.

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Property	Description
clarity.username	Username to be used when communicating with Clarity LIMS.
clarity.password	Encrypted password to be used when communicating with Clarity LIMS.
security.signing-key	Private key that should be used when signing/validating OAuth tokens. Changing this invalidates any issued tokens.
security.token-expiry	Specifies (in hours) the expiry period for login tokens issued by the Sequencer API. Default value: 88
novaseq.sequenceStepNames	List of NovaSeq sequencing run step names for which the integration may find samples queued. • Names must be an exact match to the name of the step in Clarity LIMS (not the master step/process type). • Each line should be indented to the same point and start with a dash (-). Default value: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.1)

novaseq.flowcells	List of supported NovaSeq flow cell types.
	• Type names exactly match the values used by the NVCS run status requests.
	• To add more types, provide both type and lanes properties, indented to the same point and starting with a dash (-).
	Default value:
	`- type: "SP"`
	`lanes: 2`
	`- type: "S1"`
	`lanes: 2`
	`- type: "S2"`
	`lanes: 2`
	`- type: "S4`
	`"lanes: 4`

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Property	Description
novaseq.reagents	List of supported reagent kits to be tracked on sequencing steps. - lotName: The name of the lot as it appears in the run started request. - kitName: The name of the corresponding reagent kit in Clarity LIMS that the lot should be tracked as. • The name may differ in the NVCS request vs. the Clarity LIMS configuration. Default value: `- lotName: "Buffer"kitName: "Buffer Cartridge"- lotName: "Cluster"kitName: "Cluster Cartridge"- lotName: "SBS"kitName: "SBS Cartridge"`
recipe.udfNames	Configures the names of the fields used in the run recipe. See *Run Recipe Contents* section for more information). • Each value must be enclosed in quotes. Example: recipe.udfNames.runMode "Run Mode" • Value provided for sampleLoadingType must match the value provided in the run configuration. Example: recipe.udfNames.sampleLoadingType "Loading Workflow Type"
recipe.sampleSheet.outputName recipe.sampleSheet.notAvailableValue	Configures the name of the sample sheet file placeholder on the step where run recipe information is populated, and the property value to use when the file is not found.
run.stepUdfNames	Configures the step UDF/custom field names to be used when recording run results. • Each value must be enclosed in quotes. Example: run.stepUdfNames.runID "Run ID" • Value provided for sampleLoadingType must match the value provided in the run configuration. • Example: run.stepUdfNames.sampleLoadingType "Loading Workflow Type". It is expected that the run step field and the field on the input derived sample share a name.

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Property	Description
run.metricUdfNames	Configures the result file UDFs/measurement global field names to be used when recording run results. These are the fields that capture the run data.
run.autoComplete	Determines whether the sequencing run step should be completed automatically after parsing the run metrics on a run (see also run.autoCompleteOnlyAtSuccess). Default value: true See the *Auto Complete* section for more information.
run.autoCompleteOnlyAtSuccess	Determines which of the following options the sequencing run step should be: • Autocompleted when the run status is RunCompletedSuccessfully (ifautoCompleteOnlyAtSuccess is true) • Autocompleted regardless of the run status (if autoCompleteOnlyAtSuccess is false). Default value: true See the *Auto Complete* section for more information.

Reagent Categories/Label Groups

- TruSeq HT Adapters v2 (D7-D5)

Reagent Kits

- Buffer Cartridge
- Cluster Cartridge
- DPX1
- DPX2
- DPX3
- NaOH
- Resuspension Buffer (RSB)
- SBS Cartridge
- Tris HCl

Container Types

- Library Tube
- SP
- S1
- S2

- S4

This integration supports the following items:

- Library tube with barcode provided in the format [A-Za-z]{2}[0–9]{7}-[A-Za-z]{3}
  Example: AB1234567-XYZ
- SP, S1, S2, or S4 flow cell with barcode provided in one of the following formats:
  - SP and S1 flow cell: [A-Za-z0-9]{5}DRX[XY2357]
  - S2 flow cell: [A-Za-z0-9]{5}DMX[XY2357]
  - S4 flow cell: [A-Za-z0-9]{5}DSX[XY2357]

  Example: H1991DMXX

NVCS Configuration

For details on configuring NVCS for integration with Clarity LIMS, contact the Clarity LIMS Support team.

Routing Script Requirements

The requirements for the routing script functionality are as follows.

1. On the steps that use the routing script - Define Run Format (NovaSeq 6000 v3.1) and Dilute and Denature (NovaSeq 6000 v3.1) - the Next Step for all samples must be set to Remove from workflow. This value is set by a script. The value must not change in the Assign Next Steps screen.
2. Clarity LIMS v5: In the protocol configuration screen, the following settings are required and must not be changed:
   In the Next Steps section, for the last step in the protocol, the method of assigning the next step must be set to Automatic.
3. Clarity LIMS v4: In the protocol configuration area, for all protocol steps on which the Routing Script automation is configured, the following setting is required and must not be changed:
   On the Next Steps configuration tab, the Next Steps are set by a script toggle switch must be set to Yes.

Configuring Auto Complete Properties

As of NovaSeq Integration v3.3.0, the application.yml configuration file contains two additional properties:

- run.autoComplete
- run.autoCompleteOnlyAtSuccess

These properties determine the conditions under which the last step of the NovaSeq 6000 workflow, AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.1), is automatically completed:

- Only when the run status is "RunCompletedSuccessfully" (default behavior)

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-or -

- Regardless of the run status

By default, both properties are set to true and the step only completes if the run is successful. If the run fails or is aborted, manually complete the step in Clarity LIMS.

If you would like the step to autocomplete regardless of the result of the sequencing run, you must change the autoCompleteOnlyAtSuccess property value to false.

Enabling/Disabling autoComplete Properties

1. Open the application.yml file located at /opt/gls/clarity/extensions/sequencer-api/application.yml:

```
vi /opt/gls/clarity/extensions/sequencer-api/application.yml
```

2. Edit the value of the autoCompleteOnlyAtSuccessproperty as required.
3. Save the file.

The following table shows how the combined value of the run.autoComplete and run.autoCompleteOnlyAtSuccess properties affects the autocomplete behavior of the sequencing step.

Table 196 run.autoComplete and run.autoCompleteOnlyAtSuccess Value Matrix

run.autoComplete property value	run.autoCompleteOnlyAtSuccess property value	Outcome
true (default)	true (default)	• Step autocompletes only if sequencing run is successful. • If run is not successful, step does not autocomplete and run details are recorded in Clarity LIMS.
true	false	• Step autocompletes, regardless of the run status.
false	true	• Step does not autocomplete. • Run details are recorded in Clarity LIMS.
false	false	

Rules and Constraints

- The workflow configuration contains several validation checks. To make sure that the calculations work properly, it is important that you do not disable any of this validation logic. The validation checks determine the following information:
  - Which samples, and how many, can enter each step together.
  - Which samples, and how many, can be pooled together.

- The library tube ID must be unique. There should not be multiple library tube containers in the system with the same name.
- Reagent labels (indexes) must be unique.
- Only controls are permitted as unindexed samples; all other unindexed samples and pools are not permitted.
- For sample sheet generation constraints, refer to the Bcl2fastq2 Sample Sheet Generation article.
- Do not manually start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.1) step. This step is a fully automated and the sequencing service does not update samples correctly if they have been manually started.
- For the automated run to start successfully, select Validate Run Setup and Generate Sample Sheet.

Resources

- *NovaSeq 6000 Sequencing System Guide*

NovaSeq 6000 Integration v3.3.0 Validation and Troubleshooting

This guide explains how to validate the installation of the Illumina NovaSeq 6000 Integration Package v3.3.0.

The validation process involves the following items:

- Running samples through the Library Prep Validation workflow.
  - The workflow contains a single-step protocol that models the library prep required to produce normalized libraries. At the end of the step, the normalized libraries advance to the workflow selected.
- Running normalized libraries through the NovaSeq 6000 v3.1 workflow validates the following items:
  - Successful routing of samples from the Run Format (NovaSeq 6000 v3.1) step to the NovaSeq Standard (NovaSeq 6000 v3.1) or NovaSeq Xp (NovaSeq 6000 v3.1) step.
  - Automated generation of a sample sheet for use with bcl2fastq2 v2.20 analysis software. This file automatically uploads to the sequencing system via the Sequencer API.
  - Automatic validation of run setup information. Information is uploaded to the NovaSeq Control Software (NVCS) via the Sequencer API and is used to create the run recipe and initiate the run.
  - Automated tracking of the NovaSeq sequencing run and parsing of run statistics into Clarity LIMS, via the Sequencer API.

The validation steps assume installation of the Illumina NovaSeq 6000 Integration Package v3.3.0 and the default Clarity LIMS configuration.

Validation Steps

Activate Workflow, Create Project, Add and Assign Samples

The following steps set up Clarity LIMS in preparation for running samples through the Library Prep Validation and NovaSeq 6000 v3.1 workflows.

Step 1: Activate the Workflow

In the Clarity LIMS configuration area, activate the Library Prep Validation and NovaSeq 6000 v3.1 workflows.

- For Clarity LIMS v4.x (v4.2 and later), Library Prep Validation v1.1.1 can be imported from Clarity LIMS v4 with PCWP v2.0.1 installed.
- For Clarity LIMS v5.0, follow the manual upgrade guide to upgrade the existing NovaSeq v3.0.1 workflow to the latest workflow, then proceed to the following validation steps. Contact an Illumina field service engineer for assistance with this upgrade.
- For Clarity LIMS v5.1 and later, Library Prep Validation v2.0.1 can be imported from Clarity LIMS v5.0 with IPP v2.0 installed.

Step 2: Create a Project and Add Samples

1. On the Projects & Samples screen, create a project and add samples to it.
2. Assign the samples to the Library Prep Validation workflow.

Library Prep Protocol: Library Prep Validation v1.1.1/v2.0.1

This single-step protocol models the library prep required to produce normalized libraries that are ready for the NovaSeq 6000 v3.1 workflow.

Step 1: Run Library Prep Validation v2.0.1

1. In Lab View, locate the Library Prep Validation v2.0.1 protocol.
   The Library Prep Validation v2.0.1 step opens.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Placement screen, complete the following actions:
   a. In the Placed Samples area on the right, in the Container field, scan or enter the barcode of the destination container.
   b. Select the samples from the Samples to be Placed area on the left side, and drag them to the container wells on the right side.
   c. Select Add Labels.
5. On the Add Labels screen, the Labels to Add list is populated with labels from the TruSeq HT Adapters v2 (D7-D5) label group. The group is the only label group configured in the integration and is used by default in this step.

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a. Select labels on the left side, and drag them to the container on the right side to assign them to placed samples.

b. Select Record Details.

6. On the Record Details screen, complete the following actions:

a. In the Sample Details table, enter values for Normalized Molarity. Type values individually or copy and paste to enter multiple values from a spreadsheet.

b. The Sequencing Instrument column indicates the instrument to be used for the sequencing run. Select NovaSeq 3.0.

c. Populate the fields in this column using one of the following methods:

- To populate fields individually for each sample, select from the drop-down list in each row.
- To populate all fields at one time, copy and paste from a spreadsheet.

Alternatively, select inside the table and press Ctrl + A to select all rows. At the top of the table, select Sequencing Instrument and then select NovaSeq 3.0 from the adjacent drop-down list. Select Apply.

If working with many samples, copy and paste to insert multiple values from a spreadsheet as follows.

a. Select Copy Samples to copy the sample information from Clarity LIMS to the Clipboard.

b. Open a blank Excel document and paste the contents of the clipboard into the spreadsheet.

c. In the spreadsheet, enter the Normalized Molarity and Sequencing Instrument values. Select the entire spreadsheet including the headers (Ctrl + A), and copy (Ctrl + C) the information to the Clipboard.

d. Within Clarity LIMS, select the Paste Samples button. Paste the contents of the clipboard (Ctrl + V) into the Update Table window and then select Update to paste the contents into the Sample Details table.

7. Select Next Steps.
Triggers the Next Steps automation, which sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step.

8. On the Assign Next Steps screen in the Samples table, the Next Step value for all samples is prepopulated with Remove from workflow.

Do not change this value. If Next Step is not set to Remove from workflow, the routing script does not route samples correctly.

9. Select Finish Step.
Upon exiting the step, Routing Script automation is triggered. This automation assigns samples to the first step of the NovaSeq 6000 v3.1 workflow, Define Run Format (NovaSeq 6000 v3.1). It is the only step in Protocol 1: Run Format (NovaSeq 6000 v3.1).

Protocol 1: Run Format (NovaSeq 6000 v3.1)

This protocol includes a single step, Define Run Format (NovaSeq 6000 v3.1). The step allows for the assignment of per sample values for Loading Workflow Type, Normalized Molarity, Flowcell Type, and Final Loading Concentration (pM). At the end of the step, samples are routed to the NovaSeq Standard or NovaSeq Xp protocol, according to the selected Loading Workflow Type.

Step 1: Run Define Run Format (NovaSeq 6000 v3.1)

1. In Lab View, locate the Run Format (NovaSeq 6000 v3.1) protocol. The samples are queued for the Define Run Format (NovaSeq 6000 v3.1) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Record Details screen in the Sample Details table, populate the following fields (values can vary across samples):
   - Loading Workflow Type—Select NovaSeq Standard or NovaSeq Xp from the drop-down list.
   - Flowcell Type—Select SP, S1, S2, or S4.
   - Final Loading Concentration (pM)—Select from the two preset options: 225 (for PCR-free workflows), 400 (for Nano workflows), or enter a different value.
   - Normalized Molarity (nM)—These values are copied over from the previous step. If this column is not populated during library prep, enter the values here.
5. Select Next Steps, which triggers the Next Steps automation and the following actions:
   - Sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step.
   - Calculates the Minimum Molarity.
   - Checks Normalized Molarity value. For samples with no Normalized Molarity value (in other words, empty value, not including 0), generates an error message notifying that the field cannot be empty.
   - Compares each sample Normalized Molarity value with the Minimum Molarity value.
6. On the Assign Next Steps screen:
   - In the Sample Details table, the Next Step for all samples is prepopulated with Remove from workflow, regardless of the Loading Workflow Type.

     Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not routed samples correctly.

   - For samples whose Normalized Molarity value is lower than the Minimum Molarity value, the Loading Workflow Type is set to Remove from workflow. Also, a message is recorded in the Warning field for the sample.
     At this point, there are two options:

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- Return to the Record Details screen and adjust the Normalized Molarity value so that it equals or exceeds the Minimum Molarity value. The Loading Workflow Type must be set to NovaSeq Standard or NovaSeq Xp, as applicable.
- Complete the protocol without correcting the Normalized Molarity value. In this case, the samples in question are removed from the Clarity LIMS workflow.

7. Select Finish Step. The Routing Script automation is triggered:
   - Samples whose Loading Workflow Type is set to Remove from workflow are removed from the Clarity LIMS workflow. (Occurs when the Normalized Molarity value is lower than the Minimum Molarity.)
   - Samples whose Loading Workflow Type is NovaSeq Standard are routed to the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.1) step. These steps are the first two in NovaSeq standard protocol.
   - Samples whose Loading Workflow Type is set to NovaSeq Xp are routed to the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.1) step.

Protocol 2: NovaSeq Standard (NovaSeq 6000 v3.1)

In this protocol, samples are pooled and added to the library tube in preparation for the NovaSeq run. The protocol contains two steps:

1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.1)
2. Dilute and Denature (NovaSeq 6000 v3.1)

Step 1: Run Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.1)

1. In Lab View, locate the NovaSeq Standard (NovaSeq 6000 v3.1) protocol. Samples for the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.1) step are queued.
2. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Pooling screen:
   a. Create a pool by dragging samples into the Pool Creator.

      ⚠ Only create one pool.

   b. Enter a name for the pool or accept the default name (Pool #1).
   c. Select Record Details.
      After exiting from the Pooling screen, the Validate Inputs Flowcell Type and Single Pool is triggered. The automation verifies the following items:
      - All samples in the pool have been assigned the same Flowcell Type.
      - Only one pool has been created.

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5. On the Record Details screen in the Step Details area, complete the two required fields and one optional field, as needed:
   - Number of Flowcells to Sequence—Used in volume calculations, to make sure that the volumes are sufficient for the number of times the pool is sequenced.
   - Minimum Per Sample Volume (µl)—Used to calculate how much of each sample is included in the pool. The field is prepopulated with the configured default value 5 µl, but can be edited.
   - % PhiX (2.5 nM) Spike-In—Used to calculate the volume of PhiX v3 control included in the pool for the given percentage of spike-in. The field is optional and can be edited.

   Assuming the default Minimum Per Sample Volume (µl) value of 5, for a given batch:
   - If the smallest Per Sample Volume (µl) value is < 5, Clarity LIMS automatically assigns a value of 5 to the Adjusted Per Sample Volume (µl) field.
   - Clarity LIMS changes the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.
6. In the Sample Details table, select the pool icon to view details on the pool composition.
7. Select Calculate Volumes to trigger the Calculate Volumes automation, which performs volume calculations based on the selected Flowcell Type.
   - Calculates Bulk Pool Volume (µl) value.
   - Sets the value of the Bulk Pool Volume (µl) field.
   - Calculates the PhiX Volume (µl) value if % PhiX (2.5 nM) Spike-In has value.
   - Calculates the Per Sample Volume (µl) value to be added to the pool.
   - Calculates the Total Sample Volume (µl) value.
   - If the Total Sample Volume is less than the Bulk Pool Volume, selects the RSB Volume (µl) value.
   - Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
   - Populates the Volume of Pool to Denature (µl), NaOH Volume (µl) and Tris-HCl Volume (µl) columns of the Sample Details table. The script sets the values, and is not editable.
   - The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains volume information about the pool and the individual samples it contains. Select on the file to download it and then open it in Excel.
8. Select Next Steps to trigger the Set Next Step automation. This automation sets the next step for samples to ADVANCE and advances them to the next step in the protocol, Dilute, and Denature (NovaSeq 6000 v3.1).
   On the Assign Next Steps screen, the next step for samples is set to the next step in the workflow: Dilute and Denature (NovaSeq 6000 v3.1).
9. Select Finish Step.
   At the end of this step, the pool of samples automatically advances to the Dilute and Denature (NovaSeq 6000 v3.1) step.

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Step 2: Run Dilute and Denature (NovaSeq 6000 v3.1)

1. In Lab View, locate the NovaSeq Standard (NovaSeq 6000 v3.1) protocol. The pool of samples queued for the Dilute and Denature (NovaSeq 6000 v3.1) step are listed.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work. The Validate Single Input automation is triggered. This automation verifies that only one container is input for the step.
4. On the Placement screen:
   a. Drag the pool into the library tube in the Placed Samples area.
   b. Scan or type the barcode of the library tube into the Library Tube field.
   c. Select Record Details.
   After exiting the Placement screen, the Validate Library Tube Barcode automation makes sure that the library tube barcode conforms to the barcode mask [A-Za-z]{2}[0-9]{7}-[A-Za-z]{3}. If it does not, an error message displays. The automation also copies the Flowcell Type and Loading Workflow Type values from step inputs to outputs.
5. On the Record Details screen, the Reagent Lot Tracking section lets you tracks the NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. Add and activate lots for these reagents.
   To add and activate reagent lots:
   a. In a new browser tab, open an additional instance of Clarity LIMS.
      - LIMS v5: Navigate to the Consumables configuration screen.
      - LIMS v4: Navigate to the Reagents and Controls screen.
   b. Select the NaOH reagent kit and select New Lot.
   c. Enter the lot details and select Save.
   d. Set the Status of Reagent Lot toggle switch to Active.
   e. Repeat this process to add and activate Resuspension Buffer and Tris-HCl reagent lots.
   f. Return to your original working browser tab and refresh the page.
6. In the Reagent Lot Tracking section, select from the active lots displayed in each drop-down list.
7. The fields displayed on the Record Details screen are used to set up the run and generate the sample sheet. Some of these fields are autopopulated and some must be completed manually.

Table 197 Fields Displayed on Record Details Screen of Denature and Dilute (NovaSeq 6000 v3.1)

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.
BaseSpace Sequence Hub Configuration	Select from preset options: Not Used, Run Monitoring Only, or Run Monitoring and Storage.

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Field	Value
Workflow	Automatically populated with the preset GenerateFASTQ.
Workflow Type	Select from preset options: No Index, Single Index, Dual Index, or Custom.
Index Read 1	Select from preset options: 0, 6, or 8, or type a value between 0 and 20.
Index Read 2	Select from preset options: 0, 6, or 8, or type a value between 0 and 20.
Paired End	Select from preset options: True or False.
Read 1 Cycles	Select from preset options: 251, 151, 101, or 51, or type a value between 1 and 251. *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Read 2 Cycles	Select from preset options: 251, 151, 101, or 51, or type a value between 1 and 251. *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Use Custom Read 1 Primer	Select if applicable.
Use Custom Read 2 Primer	Select if applicable.
Use Custom Index Read 1 Primer	Select if applicable.
Reverse Complement Workflow	Select Yes (by default) for v1.5 reagents in NovaSeq Control Software v1.7 and onwards. Select No for v1 reagents.
Use Custom Recipe	Select if applicable.
Custom Recipe Path	If the Use Custom Recipe option is selected, enter the path to the custom recipe file to be used.
UMI - Read 1 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 1. Leave blank if not applicable.
UMI - Read 2 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 2. Leave blank if not applicable.
UMI - Read 1 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.
UMI - Read 2 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.

Field	Value
Paired End	Select if applicable.
Output Folder	Enter network path for sequencing run folder. For example: \\networkshare\run_data

8. On the Record Details screen, select Validate Run Setup and Generate Sample Sheet. This triggers the automation script, which does the following actions:
   - Validates the parameters entered on the Record Details screen.
   - Generates the sample sheet and attaches it to the placeholder in the Files area of the Record Details screen.
9. Select Next Steps.
   On the Assign Next Steps screen, the Next Step for samples is prepopulated with Remove from workflow. The Routing Script automation expects this value, and requires the value to advance samples to the next step.

   Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.
10. Select Finish Step.
    After exiting the step, the following actions occur:
    - The Routing Script automation is triggered and samples are routed to AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.1).
    - In Lab View, the pool of samples is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.1) step.

    At this point in the workflow, the user interaction ends. Proceed to NovaSeq Run (NovaSeq 6000 v3.1).

    Do not add samples to the Ice Bucket or start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.1) step. The integration adds samples automatically.

Protocol 3: NovaSeq Xp (NovaSeq 6000 v3.1)

In this protocol, samples are pooled and added to lanes on the NovaSeq flow cell. The flow cell type is determined by the option selected in the Define Run Format (NovaSeq 6000 v3.1) step.

The protocol contains three steps:

1. Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.1)
2. Dilute, Denature, and ExAmp (NovaSeq 6000 v3.1)
3. Load to Flowcell (NovaSeq 6000 v3.1)

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Step 1: Run Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.1)

1. In Lab View, locate the NovaSeq Standard (NovaSeq 6000 v3.1) protocol. The samples are queued for the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.1) step.
2. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Pooling screen:
   a. Create a pool by dragging samples into the Pool Creator.

      Create only one pool.

   b. Enter a name for the pool or accept the default name (Pool #1).
   c. Select Record Details.
5. On exiting the Pooling screen, the Validate Inputs Flowcell Type and Single Pool automation is triggered. The automation verifies the following items:
   - All samples in the pool are assigned the same Flowcell Type.
   - Only one pool was created.
6. On the Record Details screen in the Step Details area, complete the two required fields and one optional field, as needed:
   - Number of Lanes to Sequence—Used in volume calculations to make sure that volumes are sufficient for the number of times the pool is sequenced.
   - Minimum Per Sample Volume (µl)—Used to calculate how much of each sample is included in the pool. The field is prepopulated with the configured default value 5 µl, but can be edited.
   - % PhiX (0.25nM) Spike-In—Used to calculate the volume of PhiX v3 control to be included in the pool for the given percentage of spike-in. The field is optional and can be edited.

   Assuming the default Minimum Per Sample Volume (µl) value of 5, for a given batch:
   - If the smallest Per Sample Volume (µl) value is < 5, Clarity LIMS automatically assigns a value of 5 to the samples Adjusted Per Sample Volume (µl) field.
   - Clarity LIMS then adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.
7. In the Sample Details table, select the pool icon to view details on the pool composition.
8. Select Calculate Volumes. Triggers the Calculate Volumes automation. This automation performs volume calculations based on the selected Flowcell Type, as follows.
   - Calculates Bulk Pool Volume (µl) value.
   - Calculates the Per Sample Volume (µl) value to be added to the pool.
   - Calculates the Total Sample Volume (µl) value.

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- If the Total Sample Volume is less than the Bulk Pool Volume, calculate the RSB Volume (µl) value.
- The automation also populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
- The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains volume information about the samples and RSB buffer to add to the pool. Select the file to download it and then open it in Excel.

9. Select Next Steps to trigger the Set Next Step automation. This automation performs the following actions:
    - Copies the Flowcell Type values from the step inputs to the step outputs.
    - Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol Dilute, Denature, and ExAmp (NovaSeq 6000 v3.1).

    On the Assign Next Steps screen, the next step for samples is set to the next step in the workflow Dilute, Denature, and ExAmp (NovaSeq 6000 v3.1).
10. Select Finish Step.
    At the end of this step, the pool of samples automatically advances to the Dilute, Denature, and ExAmp (NovaSeq 6000 v3.1) step.

Step 2: Run Dilute, Denature and ExAmp (NovaSeq 6000 v3.1)

1. In Lab View, locate the NovaSeq Xp (NovaSeq 6000 v3.1) protocol. The pool of samples queued for the Dilute, Denature, and ExAmp (NovaSeq 6000 v3.1) step displays.
2. Add the pool to the Ice Bucket and select View Ice Bucket.
3. [Optional] On the Ice Bucket screen, set the number of derivatives to create (placed into the flow cell lanes) and select Begin Work.
4. On entry to the Record Details screen, the Calculate Volumes automation is triggered.
    This automation sets the following values based on the selected Flowcell Type:
    - BP Aliquot Volume (µl)
    - NaOH Volume (µl)
    - Tris-HCl Volume (µl)
    - DPX1 Volume (µl)
    - DPX2 Volume (µl)
    - DPX3 Volume (µl)
    - Mastermix per Lane (µl)
    - The automation also populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.

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- The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains information about the DPX Mastermix volume and the volume of Mastermix, NaOH, and Tris-HCl to add per working pool (see the next step).

5. On the Record Details screen, the Reagent Lot Tracking section tracks the DPX1, DPX2, DPX3, NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. These reagents must be added.
   To add and activate reagent lots:
   a. In a new browser tab, open an additional instance of Clarity LIMS.
      - Clarity LIMS v5: Navigate to the Consumables configuration screen.
      - Clarity LIMS v4: Navigate to the Reagents and Controls screen.
   b. Select one of the reagent kits previously listed and select New Lot.
   c. Enter the lot details and select Save.
   d. Set the Status of Reagent Lot toggle switch to Active.
   e. Repeat this process to add and activate the remaining reagent lots.
   f. Return to your original working browser tab and refresh the page.
6. On the Record Details screen:
   - In the Reagent Lot Tracking section, select from the active lots displayed in each drop-down list.
   - In the Step Details area, the DPX1, DPX2, and DPX3 reagent volume values are prepopulated. The script sets the values and they are not editable.
   - In the Sample Details table, select the pool icon to view details on the working pool composition.
     - The BP Aliquot, Mastermix per lane, NaOH, and Tris-HCl volume values for each working pool are populated. The script sets the values and they are not editable.
     - The Flowcell Type and Loading Workflow Type columns are populated.
     - The working pool number is appended to the bulk pool name, to identify which working pools are derived from the same bulk pool.
7. In the Files area, select the Calculation File (CSV) to open it and view details on the DPX Mastermix volume and the volume of Mastermix, NaOH, and Tris-HCl to add per working pool.
8. Select Next Steps.
   On the Assign Next Steps screen, the next step is already set to Load to Flowcell (NovaSeq 6000 v3.1).
9. Select Finish Step.

Step 3: Run Load to Flowcell (NovaSeq 6000 v3.1)

1. On the Ice Bucket screen:
   - In the Container Options panel, select the appropriate flow cell type from the Destination Container drop-down list.

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- Select Begin Work.

2. The Validate Inputs and Selected Container automation checks the following:
   - The Flowcell Type field is set to a valid value (SP, S1, S2, or S4) and that each input has the same value for the field.
   - The container type selected matches the value in the Flowcell Type field.
   - The number of outputs matches the number of lanes on the selected flow cell type. If validation fails, an error message reports the number of working pools does not match the number of lanes available on the flow cell.
3. On the Placement screen:
   - Drag one or more pools from the left of the screen over into the Placed Samples area on the right.
   - Scan or type the barcode of the flow cell into the Flowcell field.
   - Select Record Details.

   After exiting the Placement screen, the Validate Flowcell Barcode automation makes sure that the container barcode conforms to the barcode mask for the selected Flowcell Type (as listed in the following table). This automation also copies the Flowcell Type and Loading Workflow Type field values from step inputs to outputs.

Flowcell Type	Barcode Mask
SP	[A-Za-z0-9]{5}DRX[XY2357]
S1	[A-Za-z0-9]{5}DRX[XY2357]
S2	[A-Za-z0-9]{5}DMX[XY2357]
S4	[A-Za-z0-9]{5}DSX[XY2357]

4. The fields displayed on the Record Details screen are used to set up the run and generate the sample sheet.
   Some of these fields are autopopulated and some must be entered manually (see the following table for details).

Table 198 Fields Displayed on Record Details Screen of Load to Flowcell (NovaSeq 6000 v3.1) Step

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. No spaces.
BaseSpace Sequence Hub Configuration	Select from preset options: Not Used, Run Monitoring Only, or Run Monitoring and Storage.

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Field	Value
Workflow	Automatically populated with preset - GenerateFASTQ.
Workflow Type	Select from preset options: No Index, Single Index, Dual Index, or Custom.
Index Read 1	Select from preset options: 0, 6, or 8 - or type a value between 0 and 20.
Index Read 2	Select from preset options: 0, 6, or 8 - or type a value between 0 and 20.
Paired End	Select from preset options: True or False.
Read 1 Cycles	Select from preset options: 251, 151, 101, or 51 - or type a value between 1 and 251. *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Read 2 Cycles	Select from preset options: 251, 151, 101, or 51 - or type a value between 1 and 251. *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Use Custom Read 1 Primer	Select if applicable.
Use Custom Read 2 Primer	Select if applicable.
Use Custom Index Read 1 Primer	Select if applicable.
Reverse Compliment Workflow	Select Yes (by default) for v1.5 reagents in NovaSeq Control Software v1.7 and onwards. Select No for v1 reagents.
Use Custom Recipe	Select if applicable.
Custom Recipe Path	If you selected the Use Custom Recipe option, enter the path to the custom recipe file to be used.
UMI - Read 1 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 1. Leave blank if not applicable.
UMI - Read 2 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 2. Leave blank if not applicable.
UMI - Read 1 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.
UMI - Read 2 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.

Field	Value
Output Folder	Enter network path for sequencing run folder. For example: \\networkshare\run_data
Library Tube Barcode	Scan the library tube barcode.

5. Select Validate Run Setup and Generate Sample Sheet, then select Next Steps to trigger the automation script, which:
   - Validates the parameters entered on the Record Details screen.
   - Generates the sample sheet and attaches it to the placeholder in the Files area on the Record Details screen.
6. Select Next Steps.
   On the Assign Next Steps screen, the Next Step field for samples is prepopulated with Mark protocol as complete.
7. Select Finish Step.
   At this point in the workflow, the user interaction ends. The flow cell is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.1) step.

Proceed to AUTOMATED - .NovaSeq Run (NovaSeq 6000 v3.1).

Do not add samples to the Ice Bucket or start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.1) step. The integration adds samples automatically.

Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.1)

This protocol contains a single fully automated step - AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.1).

The integration starts the step automatically and data from the run is parsed back into Clarity LIMS. User interaction is not required, but the various stages can be reviewed in Clarity LIMS (see the Review Run Data section).

Automated - NovaSeq Run (NovaSeq 6000 v3.1) contains two automations:

1. Set Next Steps automation is disabled (Set to Not Used). By default, the Sequencer API is used to set the next step for output samples.
   - The PCWP includes a Set Next Steps automation configured on the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.1) step. This automation can be used to override the default next step behavior. See NovaSeq 6000 User and Configuration Guide for details.
2. Update Lane Number automation is triggered automatically upon entry to the Record Details screen.
   - Do not disable or modify this automation to make sure that the Lane Number is displayed properly.

Review Run Data

Read summary metrics are recorded for the library pool. After the run is complete, open the step and review these metrics on the Next Steps screen in the Step Data section and the Sample Details table.

Step Data Section

The following values populate in the step UDFs/master step fields:

- Run ID
- Run Status
- Output Folder
- Current Cycle
- Current Read
- Loading Workflow Type
- Flow Cell ID
- Flow Cell Side
- Flow Cell Mode
- Flow Cell Part Number*
- Flow Cell Lot Number
- Flow Cell Expiration Date
- Instrument ID
- Instrument Type
- Instrument Control Software Version
- Firmware Version
- RTA Version
- Sequencing Log

Sample Details Table

Summary metrics populate the UDFs/global fields listed. Values are aggregated across all lanes. Some values (for example Yield PF (Gb) R1) are summed, while others are averaged.

- Yield PF (Gb) R1
- Yield PF (Gb) R2*
- Reads PF (M) R1*
- Reads PF (M) R2*
- Cluster Density (K/mm^2) R1

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- Cluster Density (K/mm^2) R2
- %PF R1
- %PF R2
- % Bases >=Q30 R1
- % Bases >=Q30 R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- % Aligned R1
- % Aligned R2
- % Error Rate R1
- % Error Rate R2

For R2 Yield, R2 Error rate, and R2 Q30, there are discrepancies between the values shown in Illumina SAV software and the payload sent from the NVCS to Clarity LIMS. This issue is fixed in the upcoming NVCS release.

How the Integration Works

The following steps summarize how the Sequencer API integration works. See NovaSeq 6000 User and Configuration Guide for details.

1. When setting up a run on the NovaSeq instrument, NVCS sends a request for the run recipe.
   a. The Sequencer API validates that samples and containers are correctly queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.1 step.
   b. Clarity LIMS sends a JSON response to NVCS. The response includes the run recipe information and a link to download the sample sheet (used with analysis software such as bcl2fastq2).
2. When the run starts, NVCS sends a RunStarted run status request. The Sequencer API then does the following:
   a. Validates reagent kit information in the RunStarted request:
      - Verifies that the reagent kit exists. If the reagent kit does not exist, the Sequencer API creates and enables it on the master step.
      - Verifies that the reagent kit is activated. If the reagent kit is not active, the Sequencer API activates it.

b. Initiates the step for the queued samples. The step produces one output file placeholder per lane of the flow cell in use, based on the flow cell type.

c. Records all relevant information from the RunStarted request on the step (such as reagent lots and step fields).

3. At the end of the run, NVCS sends one of the following run status requests:
   - RunCompletedSuccessfully
   - RunEndedByUSer
   - RunErroredOut

   The Sequencer API updates the step with any new information, which includes only the status and cycle/read information.

4. After primary analysis completes, NVCS sends a request containing the parsed run metrics. NVCS sends a request only when the run completed successfully, but the API does not make this assumption and accepts the request regardless of status. The Sequencer API then does the following:

   a. Records the metrics into the fields on file placeholder outputs in the LIMS.

   b. Completes the step in Clarity LIMS if the status is RunCompletedSuccessfully. For all other status options, the step remains in progress. This action is the default autocomplete step behavior. See NovaSeq 6000 User and Configuration Guide for details.

   > The NovaSeq 6000 User and Configuration Guide provides detailed information on how the following components work with the Sequencer API integration:
   >
   > - Sample sheet generation and contents
   > - NovaSeq run
   > - User authentication and login
   > - Run recipe contents

Troubleshooting

If an automation trigger does not appear to run its corresponding scripts, see the following articles:

- Troubleshooting Automated Informatics.
- Troubleshooting EPP/Automation in the API

If an error occurs that does not provide direction on how to proceed, complete the following steps:

1. Confirm the version of the installed Illumina NovaSeq Integration Package by running the following command on the Clarity LIMS server command line:

```
rpm -qa | grep -i novaseq
```

2. If the error is related to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.1) step, review the log file information. See *Logging* on page 712 for more details.

If the automated run step starts, but does not complete:

1. Use one of the following methods to open the in progress step in Clarity LIMS:
   - Log in to the default user account.
   - In Lab View, find the step in the Recent Activities pane.
   - Search for the step in Clarity LIMS, using either the Library Tube, or Flowcell barcode as the search term. The steps depend on whether the search is a NovaSeq Standard or NovaSeq Xp run.
2. On the Record Details screen, the Sequencing Log multiline text field contains logging information. If unable to reach the Record Details screen, or if the Sequencing Log field does not contain enough information to resolve the issue, review the sequencer-api.log file, described in the following sections.
3. Contact the Clarity LIMS support team, supplying the relevant information from the troubleshooting steps already performed.

Logging

In addition to updating the Sequencing Log multiline text field on the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.1) step, the Sequencer API writes a detailed log file to /opt/gls/clarity/tomcat/current/logs/sequencer-api.log.

Log messages include the Library Tube ID and Flowcell ID whenever the messages are related to a sequencing run-related request. In the LIMS workflows, these IDs are recorded as container names.

Some log messages are not directly related to a sequencing run request, such as downloading the sample sheet. In this case, the file LIMS ID and file name are included in log messages. The sample sheet downloaded by the sequencing system has a name that includes the sequencing container ID (Library Tube for Standard or Flowcell for XP). The relevant container name search finds most the log messages for this request. However, if all messages for downloading a file are found, then the LIMS ID of the file must be known. The LIMS ID is found in the Clarity LIMS API and, for sample sheets used in a run, in the recipe response (which can also be found in the log file).

Example lines from the log file:

```
2018-06-19 21:32:19.705 INFO --- [] SequencerAPIApplication : Started
SequencerAPIApplication in 7.026 seconds (JVM running for 15.964)
...
2018-06-19 21:34:44.564 INFO --- [Library Tube Id=NV1234567-LIB, Flow Cell
Id=RM123DSXX] LimsApiLookupUtil : Searching for containers with name 'NV1234567-
LIB' or 'RM123DSXX'
....
2018-06-19 21:35:27.158 INFO --- [] FileService : Successfully retrieved file
'NV1234567-LIB.csv' with LIMS ID 40-51 through the API.
```

NovaSeq 6000 Integration v3.3.0 Release Notes

These Release Notes detail the key changes to software components for the BaseSpace Clarity LIMS NovaSeq 6000 Integration Package since version 3.2 of that package.

This is an optional software update for customers interested in the new features available with this new release.

If you are upgrading from a version prior to version 3.2 please review the release notes for version 3.2 for a list of features and bug fixes introduced in that version.

Compatibility

- Clarity LIMS v4.2 through v5.4
- NGS Extensions Package v5.19 or later
- Preconfigured Workflow Package (PCWP) v2.0.1 or later
- Illumina Preset Protocols (IPP) v2.0.0 or later
- NovaSeq Control Software (NVCS) v1.7.0
- Illumina Bcl2FastQ v2.20

New Features

This release of the Clarity LIMS integration package:

- Supports the security improvements made to the management of passwords in Clarity LIMS v5.4.
- Provides a PhiX spike-in volume calculation script.
- Makes the ReadsPFR1 and ReadsPFR2 available for use.
- Provides error logging enhancements.
- Delivers an updated workflow (NovaSeq 6000 v3.1) directly from PCWP v2.0.1 or IPP v2.0.0.

Defect Repairs

The hard dependency on PCWP version 1.0 is corrected, allowing the use of later versions of PCWP.

Known Issues

None

NovaSeq 6000 Integration v3.2.2 Configuration and User Interaction

The Illumina NovaSeq 6000 Integration Package v3.2.2 supports the integration of Clarity LIMS to Illumina NovaSeq 6000 instruments.

Note the following key changes for this version of the integration:

- Release of the NovaSeq v3.0.1 workflow to support NVCS 1.7 and v1.5 reagents.
- A workflow as part of the Preconfigured Workflows Package (PCWP) v1.2.2.

Configuration changes in v3.2.2 include the following items:

- Support for both Reverse Complement and Forward Workflows
- Support for Unique Molecular Identifiers (UMI)

This user and configuration guide describes the integration and includes information about the preconfigured workflows, protocols, steps, and automations. The guide also lists installed components, and provides configuration requirements, rules, and constraints.

For instructions on validating and troubleshooting the Illumina NovaSeq 6000 Integration, see the *NovaSeq Integration v3.2.2 Validation and Troubleshooting Guide*.

The configuration provided in this integration has been established to support NovaSeq 6000 lab processes. Any configuration changes to protocols or workflows—including renaming protocols, steps, and fields—could break processes.

Integration Overview

The integration includes the following:

- Preconfigured NovaSeq 6000 v3.0.1 workflow that maps to lab protocols and instrument runs.
- Preconfigured protocols:
  - Run Format (NovaSeq 6000 v3.0.1)
  - NovaSeq Standard (NovaSeq 6000 v3.0.1) (supports the loading of pooled libraries into a library tube)
  - NovaSeq Xp (NovaSeq 6000 v3.0.1) (supports individual lane loading on the NovaSeq)
  - Automated—NovaSeq Run (NovaSeq 6000 v3.0.1)
- Automated generation of a sample sheet for use with bcl2fastq2 v2.20 analysis software. This file is automatically uploaded to the sequencing instrument via the Sequencer API and is used to set up the run.
- Automatic validation of run setup information. This information is used to create the run recipe and initiate the run.
- Automated tracking of the NovaSeq sequencing run and parsing of run statistics into Clarity LIMS, including:
  - Progress and metrics of sequencing run
  - Per-instrument sequencing runs (tracked as part of the Run ID field value)
  - Sequencing run parameters
  - Sequencing run data directory location
  - Real-Time Analysis v3 (RTA3) version and other run-specific information

- [Optional] Preconfigured Library Prep Validation v1.0.1 workflow used for validation purposes only. The workflow contains a single-step protocol that models the library prep required to produce normalized libraries that are ready for the NovaSeq 6000 v3.0.1 workflow. See the *NovaSeq Integration v3.2.2 Validation and Troubleshooting Guide*.

Information on these features is provided in the following sections.

Prerequisites and Assumptions

It is assumed that samples enter the NovaSeq 6000 v3.0.1 workflow as normalized libraries. That is, before they are assigned to the workflow:

- Samples have been accessioned into Clarity LIMS.
- Samples have been run through QC and library prep.
- Samples have been normalized, and the value is captured in a field called Normalized Molarity (nM).

For details on sample accessioning, see the articles in the Samples section of the Clarity LIMS documentation for your version.

Samples can be assigned to the NovaSeq 6000 v3.0 workflow automatically (using a routing script) or manually from the Projects and Samples dashboard. See the *Assigning Samples to Workflows* article in the Clarity LIMS documentation.

Workflows, Protocols, and Steps

The Illumina NovaSeq 6000 Integration Package v3.2.2 includes two workflows:

- NovaSeq 6000 v3.0.1
- Library Prep Validation v1.0.1 (optional, but recommended for validation purposes)

The following table lists and describes the protocols and steps included in these workflows.

Table 199 Table 1: Illumina NovaSeq 6000 Integration Package v3.2.2 Protocols and Steps

Library Prep Validation v1.0.1 workflow		
Protocol Name	Protocol Purpose	Steps Included in Protocol
Library Prep Validation v1.0.1	Included for validation purposes only, this protocol models the library prep steps required to advance samples to the Run Format (NovaSeq 6000 v3.0.1) protocol. The protocol contains a single step - Library Prep Validation v1.0.1. At the end of this step, a routing script sends the samples to the first step of the NovaSeq 6000 v3.0.1 workflow. The first step is Define Run Format (NovaSeq 6000 v3.0.1).	1. Library Prep Validation v1.0.1
NovaSeq 6000 v3.0 workflow		
Protocol name	Protocol purpose	Steps included in protocol
Run Format (NovaSeq 6000 v3.0.1)	Allows for the assignment of per sample values for Loading Workflow Type, Normalized Molarity, Flowcell Type, and Final Loading Concentration (pM): • Loading Workflow Type: Select from NovaSeq Standard or NovaSeq Xp. • Normalized Molarity: Enter a value for each sample. • Flowcell Type: User selects from options SP, S1, S2, or S4. • Final Loading Concentration (pM): Select from options 225 (PCR-free workflows) or400 (Nano workflows), or may enter a different value. Compares the Normalized Molarity value of each sample with the Minimum Molarity value. Routing script sends samples to the NovaSeq Standard or NovaSeq Xp protocol, according to the selected Loading Workflow Type. Samples with Normalized Molarity less than Minimum Molarity are removed from the workflow.	1. Define Run Format (NovaSeq 6000 v3.0.1)

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Library Prep Validation v1.0.1 workflow		
Protocol Name	Protocol Purpose	Steps Included in Protocol
NovaSeq 6000 v3.0 workflow		
Protocol name	Protocol purpose	Steps included in protocol
NovaSeq Standard (NovaSeq 6000 v3.0.1)	Samples are pooled and added to the library tube in preparation for the NovaSeq run. Run setup information is validated and sample sheet is generated. Routing script sends library tube to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0.1) protocol.	1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.0.1) 2. Dilute and Denature (NovaSeq 6000 v3.0.1)
NovaSeq Xp (NovaSeq 6000 v3.0.1)	Samples are pooled and added to lanes on the NovaSeq flow cell. The flow cell type is determined by the option selected in the Define Run Format (NovaSeq 6000 v3.0.1) step. Run setup information is validated and sample sheet is generated. Routing script sends flow cell to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0.1) protocol.	1. Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.0.1) 2. Dilute, Denature, and ExAmp (NovaSeq 6000 v3.0.1) 3. Load to Flowcell (NovaSeq 6000 v3.0.1)
AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0.1)	All samples complete the workflow by going through this protocol. This protocol contains one fully automated step. Do not add samples to the Ice Bucket or start the step. The integration does this action automatically.	1. AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0.1)

Validation Workflow

The Library Prep Validation v1.0.1 workflow allows for validation of the system after installation is complete. For details, see the NovaSeq Integration v3.2.2 Validation and Troubleshooting Guide.

Step Automation

The following table lists the step automation included in the NovaSeq 6000 v3.0.1 workflow. Automation details are provided in the sections following the table.

For details on the validation workflow, see the NovaSeq Integration v3.2.2 Validation and Troubleshooting Guide.

Table 200 Table 2: NovaSeq 6000 v3.0.1 Workflow Step Automations

Protocol	Step	Automation
1. Run Format (NovaSeq 6000 v3.0.1)	1. Define Run Format (NovaSeq 6000 v3.0.1)	• Routing Script • Set Next Steps
2. NovaSeq Standard (NovaSeq 6000 v3.0.1)	1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.0.1)	• Calculate Volumes • Set Next Step • Validate Inputs Flowcell Type and Single Pool • Validate Unique Indexes Not used. This functionality is handled by the core Clarity LIMS configuration for pooling.
	2. Dilute and Denature (NovaSeq 6000 v3.0.1)	• Routing Script • Check Library Tube Barcode • Validate Run Setup and Generate Sample Sheet • Validate Single Input
3. NovaSeq Xp (NovaSeq 6000 v3.0.1)	1. Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.0.1)	• Calculate Volumes • Set Next Step • Validate Inputs Flowcell Type and Single Pool • Validate Unique Indexes Not used. This functionality is handled by the core Clarity LIMS configuration for pooling.
	2. Dilute, Denature, and ExAmp (NovaSeq 6000 v3.0.1)	• Calculate Volumes • Set Next Step • Validate Inputs Flowcell Type
	3. Load to Flowcell (NovaSeq 6000 v3.0.1)	• Validate Flowcell Barcode • Validate Inputs and Selected Container • Validate Run Setup and Generate Sample Sheet

Protocol	Step	Automation
4. AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0.1)	1. AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0.1)	• Update Lane Number • Set Next Steps Not used. By default, this functionality is determined by the Sequencer API. If necessary, you can enable the automation and use it to override the next step behavior used by the Sequencer API.

Configuration Details

The following sections describe the configuration installed with the Illumina NovaSeq 6000 Integration.

The Illumina NovaSeq 6000 Integration Package v3.2.2 supports Clarity LIMS v4.2 to v5.3). Clarity LIMS v5.4 and later is not supported.

As of Clarity LIMS v5, the term user-defined field (UDF) has been replaced with custom field in the user interface (the API resource is still called UDF). There are two types of custom fields:

- Master step fields—Configured on master steps. These fields only apply to the master step on which they are configured, and the steps derived from those master steps.
- Global fields—Configured on entities such as submitted sample, derived sample, measurement, etc. These fields apply to the whole Clarity LIMS system.

Protocol 1: Run Format (NovaSeq 6000 v3.0.1)

This protocol sets the Loading Workflow Type and allows you to choose the appropriate Flowcell Type and Final Loading Concentration (pM). At the end of the protocol, a routing script sends the normalized libraries to either the NovaSeq Standard (NovaSeq 6000 v3.0.1) or the NovaSeq Xp (NovaSeq 6000 v3.0.1) protocol.

This protocol contains one step: Define Run Format (NovaSeq 6000 v3.0.1), described in the following section.

Step 1. Define Run Format (NovaSeq 6000 v3.0.1)

Step input: NTP (normalized libraries)

Step output: None

The following automations are configured on this step:

- Routing Script
- Set Next Steps
- Define Run Format (NovaSeq 6000 v3.0.1) automation configuration - Clarity LIMS v5

These automations are described in the order in which they are triggered at run time.

Set Next Steps

Automatically triggered on exit of the Record Details screen, this automation does the following actions:

- Sets the next step for samples to REMOVE:

```
nextStep = ::REMOVE::
```

- Calculates the Minimum Molarity using the following formula:

```
input.::Minimum Molarity (nM):: = (5 * input.::Final Loading Concentration
(pM)::)/1000
```

- Checks Normalized Molarity value. For samples with no Normalized Molarity value (ie, an empty value, not including 0), it generates an error message informing that the field cannot be empty:

```
if (!input.hasValue(::Normalized Molarity (nM)::)) { \
  fail(::The Normalized Molarity cannot be empty.::) ;
}
```

- Compares the Normalized Molarity value of each sample with the Minimum Molarity value. If the Normalized Molarity value is lower than the Minimum Molarity value, sets the Loading Workflow Type of the sample to Remove from workflow. The automation also records a message in the Warning field for the sample:

```
else if (input.::Normalized Molarity (nM):: < input.::Minimum Molarity (nM)::)
{ \
  input.::Warning:: = ::The Normalized Molarity is too low.:: ; \
  input.::Loading Workflow Type:: = ::[Remove from workflow]:: ; } \
else { input.::Warning:: = ::n/a:: }
```

At this point, there are two options:

- Correct the Normalized Molarity value on the Record Details screen. Edit the Loading Workflow Type field and set it to NovaSeq Standard or NovaSeq Xp, as applicable.
- Complete the protocol without correcting the Normalized Molarity value. In this case, those samples are removed from the workflow.

Routing Script

Automatically triggered on exit of the step, this automation invokes the changeWorkflow script, which routes step inputs appropriately.

- Samples with Loading Workflow Type field value = NovaSeq Standard are routed to the NovaSeq 6000 v3.0.1 workflow and queued for the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.0.1) step.
- Samples with Loading Workflow Type field value = NovaSeq Xp are routed to the NovaSeq 6000 v3.0.1 workflow and queued for the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.0.1) step.

The default automation command line is as follows.

```
bash -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -u {username} -p {password} -i {stepURI:v2} -l
{compoundOutputFileLuid0} script:changeWorkflow \\
--FIELD_NAME 'Loading Workflow Type' \
--FIELD_VALUE 'NovaSeq Standard' \
--WORKFLOW 'NovaSeq 6000 v3.0.1' \
--STEP 'Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.0.1)' \
--INPUTS_OR_OUTPUTS 'INPUTS' \\
--FIELD_NAME 'Loading Workflow Type' \
--FIELD_VALUE 'NovaSeq Xp' \--WORKFLOW 'NovaSeq 6000 v3.0.1' \
--STEP 'Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.0.1)' \
--INPUTS_OR_OUTPUTS 'INPUTS'"
```

Step UDFs/Custom Fields

There are four fields defined on the Define Run Format (NovaSeq 6000 v3.0.1) step.

The fields are shown in the Clarity LIMS v5 Configuration area (Custom Fields > Master Step Fields tab). See Table 3 for Clarity LIMS v4 and Clarity LIMS v5 field configuration details.

Table 201 Table 3: Define Run Format (NovaSeq 6000 v3.0.1) Step UDF/Custom Field Configuration

Field Name	Field Type	Field Constraints	Preset Values/Additional Options and Drop-Down Items
Comment	Multiline Text	• Required: No • Read Only: No	Not applicable
Flowcell Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• SP • S1 • S2 • S4
Instruction	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	• Default: Add Flowcell Type and Loading Workflow Type below.
Loading Workflow Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp

Global UDFs/Custom Fields

Table 4 lists the global UDFs/custom fields that are configured to display on the Define Run Format (NovaSeq 6000 v3.0.1) step.

Table 202 Table 4: Global UDF/Custom Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Configured on Analyte in Clarity LIMS v4 and Derived Sample in Clarity LIMS v5:			
Adjusted Per Sample Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Final Loading Concentration (pM)	Numeric (v4) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 225 • 400 • Decimal Places Displayed: 0
Flowcell Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• SP • S1 • S2 • S4
Loading Workflow Type	Single-line Text (v4) Text Dropdown (v5.x)	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • Remove from workflow
Minimum Molarity (nM)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2
Normalized Molarity (nM)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2
Per Sample Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Warning	Single-line Text (v4) Text Dropdown (v5)	• Required: No • Read Only: Yes • Custom Entries: Yes	• The Normalized Molarity (nM) is too low. • Not applicable

User Interaction

1. On the Record Details screen, the Sample Details table contains the following fields that must be completed (values can vary across samples):
   - Loading Workflow Type: Select NovaSeq Standard or NovaSeq Xp from the drop-down list.

- Flowcell Type: Select SP, S1, S2, or S4 from the drop-down list.
- Final Loading Concentration (pM): Select from the two preset options—225 (for PCR-free workflows) or 400 (for Nano workflows)—or may enter a different value.
- Normalized Molarity (nM): These values are copied over from the previous step. If you did not populate this column during library prep, enter the values here.

2. Select Next Steps. This selection triggers the Next Steps automation, which does the following actions:
   - Sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to successfully advance samples to the next step.
   - Calculates the Minimum Molarity.
   - Checks Normalized Molarity value. For samples with no Normalized Molarity value (ie, an empty value, not including 0), it generates an error message saying that the field cannot be empty.
   - Compares the Normalized Molarity value of each sample with the Minimum Molarity value.
3. On the Assign Next Steps screen in the Sample Details table, the Next Step for all samples is prepopulated with Remove from workflow, regardless of the Loading Workflow Type.

   Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.

   For samples whose Normalized Molarity value is found to be lower than the Minimum Molarity value, the Loading Workflow Type is set to Remove from workflow. A message is recorded in the Warning field for the sample.
   At this point, there are two options:
   - Return to the Record Details screen and adjust the Normalized Molarity value so that it equals or exceeds the Minimum Molarity value. Also, set the Loading Workflow Type to NovaSeq Standard.
   - Complete the protocol without correcting the Normalized Molarity value. In this case, the samples in question are removed from the Clarity LIMS workflow.
4. Select Finish Step. The Routing Script automation is triggered:
   - Samples whose Loading Workflow Type is set to Remove from workflow (ie, where Normalized Molarity value is lower than the Minimum Molarity) are removed from the Clarity LIMS workflow.
   - Samples whose Loading Workflow Type is set to NovaSeq Standard are routed to the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.0.1) step. This step is the first of two steps in the NovaSeq Standard (NovaSeq 6000 v3.0.1) protocol.
   - Samples whose Loading Workflow Type is set to NovaSeq Xp are routed to the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.0.1) step. This step is the first of three steps in the NovaSeq Xp (NovaSeq 6000 v3.0.1) protocol.

Protocol 2: NovaSeq Standard (NovaSeq 6000 v3.0.1)

Samples are routed to this protocol if their Loading Workflow Type value is set to NovaSeq Standard. Samples are pooled and added to a library tube in preparation for the NovaSeq run.

At the end of this protocol, a routing script sends the library tube to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0.1) protocol.

This protocol contains two steps:

- Step 1: Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.0.1)
- Step 2: Dilute and Denature (NovaSeq 6000 v3.0.1)

Step 1: Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.0.1)

In this step, place libraries into a single pool. Add resuspension buffer and reagents.

Only create one pool per step.

Step input: NTP (normalized libraries)

Step output: Bulk pool

The following automations are configured on the step:

- Calculate Volumes
- Set Next Step
- Validate Inputs Flowcell Type and Single Pool
- Validate Unique Indexes (Not used. This functionality is handled by the core Clarity LIMS configuration for pooling.)
- Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.0.1) automation configuration - Clarity LIMS v5.

These automations are described in the order in which they are triggered at run time.

Validate Inputs Flowcell Type and Single Pool

Automatically triggered on exit of the Pooling screen, this automation does the following actions:

- Checks that all samples in the pool have the same Flowcell Type assigned to them.
- Checks that only one pool has been created. Changes the value of error and logging messages to reference the type of pool passed as 'bulk' pool.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar \script:validate_flowcell_for_input_pools \
  -i {stepURI:v2} \
  -u {username} \
  -p {password} \
```


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```
  -l {compoundOutputFileLuid1} \
  -validateSingleOutput true \
  -poolType bulk"
```

Calculate Volumes

Automatically triggered when the Calculate Volumes button on the Record Details screen is selected, this automation does the following actions:

- Calculates the number of samples in the pool:

```
-exp 'step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: +
1'
```

- Sets the value of the Bulk Pool Volume (µl) field, based on the selected Flowcell Type:

```
-exp 'if ( input.::Flowcell Type:: == ::SP:: ) {
  step.::Bulk Pool Volume (µl):: = step.::Number of Flowcells to Sequence:: *
100 + 30
} ;

if ( input.::Flowcell Type:: == ::S1:: ) {
  step.::Bulk Pool Volume (µl):: = step.::Number of Flowcells to Sequence:: *
100 + 30
} ;
if ( input.::Flowcell Type:: == ::S2:: ) {
  step.::Bulk Pool Volume (µl):: = step.::Number of Flowcells to Sequence:: *
150 + 30
} ;
if ( input.::Flowcell Type:: == ::S4:: ) {
  step.::Bulk Pool Volume (µl):: = step.::Number of Flowcells to Sequence:: *
310 + 30
} ;
```

- Calculates the Per Sample Volume (µl) to be added to the pool:

```
input.::Per Sample Volume (µl):: = ( ( (input.::Final Loading Concentration
(pM):: * 5 / 1000) / input.::Normalized Molarity (nM):: ) * step.::Bulk Pool
Volume (µl):: ) / step.::Number of Samples in Pool::
```

> To ensure accurate pipetting of each sample in a pool for sequencing, the Per Sample Volume (µl) value must be equal to or higher than the Minimum Per Sample Volume (µl). The default value is set at 5 and may be edited.

Assuming the default Minimum Per Sample Volume (µl) value of 5, for a given batch:

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1. If the smallest Per Sample Volume (µl) value is less than 5, Clarity LIMS automatically assigns a value of 5 to the Adjusted Per Sample Volume (µl) field of the sample.
2. Clarity LIMS then adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

- Calculates the Total Sample Volume (µl) field value:

```
-exp 'step.::Total Sample Volume (µl):: = step.::Total Sample Volume (µl):: +
input.::Adjusted Per Sample Volume (µl)::'
```

- If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) field value:

```
if (step.::Total Sample Volume (µl):: >= step.::Bulk Pool Volume (µl)::)
{output.::RSB Volume (µl):: = 0} else {output.::RSB Volume (µl):: = step.::Bulk
Pool Volume (µl):: - step.::Total Sample Volume (µl)::} ;
```

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ;output.::Loading Workflow
Type:: = input.::Loading Workflow Type:: ;
```

- Sets the Volume of Pool to Denature (µl) value and calculates NaOH Volume (µl) and Tris-HCl Volume (µl) values, based on the Flowcell Type:

```
if ( input.::Flowcell Type:: == ::SP:: ) { output.::Volume of Pool to Denature
(µl):: = 100 ; output.::NaOH Volume (µl):: = 25 ; output.::Tris-HCl Volume
(µl):: = 25 } ;
if ( input.::Flowcell Type:: == ::S1:: ) { output.::Volume of Pool to Denature
(µl):: = 100 ; output.::NaOH Volume (µl):: = 25 ; output.::Tris-HCl Volume
(µl):: = 25 } ;
if ( input.::Flowcell Type:: == ::S2:: ) {output.::Volume of Pool to Denature
(µl):: = 150 ; output.::NaOH Volume (µl):: = 37.50 ; output.::Tris-HCl Volume
(µl):: = 37.50 } ;
if ( input.::Flowcell Type:: == ::S4:: ) { output.::Volume of Pool to Denature
(µl):: = 310 ; output.::NaOH Volume (µl):: = 77.50 ; output.::Tris-HCl Volume
(µl):: = 77.50 }
```

- Uses the `NovaSeq_Standard_Bulk_Pool1.csv`, `NovaSeq_Standard_Bulk_Pool2.csv`, and `NovaSeq_Standard_Bulk_Pool3.csv` template files to generate a single CSV file. The generated file contains information about the pool and the samples it contains. It is available for download on the Step Setup screen of the following step—Dilute and Denature (NovaSeq 6000 v3.0.1).

```
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} \ script:driver_file_generator \
  -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Standard_Bulk_
Pool1.csv \
```

```
  -o 1.csv \ script:driver_file_generator \
  -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Standard_Bulk_
Pool2.csv \
  -o 2.csv \ script:driver_file_generator \
  -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Standard_Bulk_
Pool3.csv \
  -o 3.csv \
&& cat 1.csv 2.csv 3.csv > {compoundOutputFileLuid0}.csv
```

Set Next Step

Automatically triggered on exit of the Record Details screen, this automation sets the next step for samples to ADVANCE. The samples advance to the next step in the protocol—Dilute and Denature (NovaSeq 6000 v3.0.1):

```
nextStep = ::ADVANCE::
```

Step UDFs/Custom Fields

There are five fields defined on the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.0.1) step.

These fields are shown in the Clarity LIMS v5 Configuration area (Custom Fields > Master Step Fields tab).

See Table 5 for Clarity LIMS v4 and Clarity LIMS v5 field configuration details.

Table 203 Table 5: Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.0.1) Step UDF/Custom Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Bulk Pool Volume (µl) (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2
Number of Flowcells to Sequence	Numeric	• Required: Yes • Read Only: No	• Range: 1 to 10 • Decimal Places Displayed: 0

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Total Sample Volume (µl) (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Default: 0 • Decimal Places Displayed: 2
Minimum Per Sample Volume (µl)	Numeric	• Required: Yes • Read Only: No	• Default: 5 • Decimal Places Displayed: 2
Number of Samples in Pool (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Default: 0 • Decimal Places Displayed: 0

Global UDFs/Custom Fields

The following table lists the global UDFs/custom fields that are configured to display on the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.0.1) step.

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Configured on Analyte in Clarity LIMS v4 and Derived Sample in Clarity LIMS v5:			
Flowcell Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• SP • S2 • S4
Loading Workflow Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
NaOH Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
RSB Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Tris-HCl Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Volume of Pool to Denature (µl) Used in Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.0.1) step only. Displays on Record Details screen and in the generated CSV file.	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0

User Interaction

1. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucket and select View Ice Bucket.
2. On the Ice Bucket screen, select Begin Work.
3. On the Pooling screen:
   a. Create a pool by dragging samples into the Pool Creator.
   b. Name the pool or accepts the default name (Pool #1).
   c. Select Record Details.

   On exit from the Pooling screen, the Validate Inputs Flowcell Type and Single Pool is triggered. The automation checks that all samples in the pool have been assigned the same Flowcell Type and only one pool has been created.
4. On the Record Details screen, the Step Details area contains two required fields:
   - Number of Flowcells to Sequence—The value entered in this field is used in volume calculations, to make sure that volumes are sufficient for the number of times the pool is sequenced.
   - Minimum Per Sample Volume (µl)—In many applications, and particularly where robotics are used, a minimum sample volume is required. The value in this field is used to calculate how much of each sample is included in the pool. The field is prepopulated with the configured default value, 5 µl, but may be edited if necessary.

   In the Sample Details table, select the pool icon to view details on the pool composition.

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5. Select Calculate Volumes. This action triggers the Calculate Volumes automation, which performs volume calculations based on the selected Flowcell Type:
    - Calculates Bulk Pool Volume (µl) value.
    - Sets the value of the Bulk Pool Volume (µl) field, based on the selected Flowcell Type.
    - Calculates the Per Sample Volume (µl) value to be added to the pool.
    - Calculates the Total Sample Volume (µl) value.
    - If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) value.
    - Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
    - Populates the Volume of Pool to Denature (µl), NaOH Volume (µl) and T ris-HCl Volume (µl) columns of the Sample Details table. (Values are set by a script and are not editable.)
    - Generates the Calculation File (*.csv) and attaches it to the step. This file contains volume information about the pool and the individual samples it contains. Select the file to download it, then open it in Excel.
6. Select Next Steps to trigger the Set Next Step automation. This automation sets the next step for samples to ADVANCE. The samples advance to the next step in the protocol: Dilute and Denature (NovaSeq 6000 v3.0.1).
   On the Assign Next Steps screen, the next step is already set to Dilute and Denature (NovaSeq 6000 v3.0.1).
7. Select Finish Step.
   At the end of this step, the pool of samples automatically advances to the Dilute and Denature (NovaSeq 6000 v3.0.1) step.

Step 2: Dilute and Denature (NovaSeq 6000 v3.0.1)

In this step, pooled samples are denatured and diluted by the addition of NaOH, Tris-HCl, and Resuspension Buffer (RSB). Samples are manually placed by into the library tube that is used in the NovaSeq run.

In addition, this step validates the run setup information and generates the sample sheet file.

Step input: Bulk pool

Step output: Library tube

The following automations are configured on the step:

- Routing Script
- Check Library Tube Barcode
- Validate Run Setup and Generate Sample Sheet
- Validate Single Input

- Dilute and Denature (NovaSeq 6000 v3.0.1) automation configuration - Clarity LIMS v5

These automations are described in the order in which they are triggered at run time.

Validate Single Input

Automatically triggered at the beginning of the step, this automation checks that there is only one container input to the step.

```
script:validateSampleCount -min 1 -max 1
```

Check Library Tube Barcode

Automatically triggered on exit of the Placement screen, this automation does the following actions:

- Validates the library tube barcode to make sure it conforms to the barcode mask [A-Za-z]{2}[0-9]{7}-[A-Za-z]{3}:

```
if (!output.container.name.matches(::[A-Za-z]{2}[0-9]{7}-[A-Za-z]{3}::)) { fail
(::Invalid Library Tube Barcode. Please verify and try again.::) }
```

- Copies Flowcell Type and Loading Workflow Type field values from step inputs to outputs:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ; output.::Loading Workflow
Type:: = input.::Loading Workflow Type::'
```

Validate Run Setup and Generate Sample Sheet

Automatically triggered when a button on the Record Details screen is selected, this automation does the following actions:

- Copies the Flowcell Type from the step input to the Run Mode field (hidden):

```
step.::Run Mode:: = input.::Flowcell Type::
```

- Validates the parameters entered on the Record Details screen, which is used to set up the run and generate the sample sheet file. See*Respond to Validation/Recipe Request call from Instrument*.
  - The Experiment Name field may only contain alphanumeric, dash, or underscore characters. Spaces are not permitted.
  - When Workflow Type = No Index, Index Read 1 must be zero. When Workflow Type = Single Index, Index Read 1 must be greater than zero, and Index Read 2 must be zero.
  - When Workflow Type = Dual Index, both Index Read 1 and Index Read 2 must be greater than zero.

```
if(!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) {fail(::Experiment
Name contains prohibited characters. Allowed characters are: a-z, A-Z, 0-9, -,
and _::)};if (step.::Workflow Type:: ==
```

```
::No Index::) {if (step.::Index Read 1:: !=0 || step.::Index Read 2:: != 0)
{fail(::Index Read 1 and Index Read 2 must be 0 ifNo Index is selected.::)}}else
if (step.::Workflow Type:: == ::Single Index::) {if (step.::Index Read 1:: ==0)
{0 || step.::Index Read 2:: != 0) {fail(::Index Read 1 must be greater than 0
and Index Read must be 0 if Single Index is selected.::)}} else {if
(step.::Index Read 1:: == 0 || step.::Index Read 2:: == 0) {
fail(::Index Read 1 and Index Read 2 must be greater than 0 if Dual Index is
selected.::)}};
```

- Checks the Paired End and Read 2 Cycles field values.
  - If Paired End = True and Read 2 Cycles value is 0, generates an error.

```
if (step.::Paired End::.toBoolean() && step.::Read 2 Cycles:: == 0) {
  fail(::Read 2 Cycles must not be zero if it is Paired End read.::)
};
```

- Validate UMI Settings
  - If UMI - Read 1 Length has value, then UMI - Read 1 Start From Cycle must have value.
  - If UMI - Read 1 Length has no value, then UMI - Read 1 Start From Cycle must not have value.
  - If UMI - Read 2 Length has value, then UMI - Read 2 Start From Cycle must have value.
  - If UMI - Read 2 Length has no value, then UMI - Read 2 Start From Cycle must not have value.
  - If UMI - Read 1 Length has not value, then UMI - Read 2 Length must not have value.
  - If UMI - Read 1 Start From Cycle has no value, then UMI - Read 2 Start From Cycle must not have value.

```
if (step.hasValue(::UMI - Read 1 Length::) && !step.hasValue(::UMI - Read 1
Start From Cycle::)) { fail(::UMI - Read 1 Start From Cycle must be greater than
0 if UMI - Read 1 Length is greater than 0.::) }; if (!step.hasValue(::UMI -
Read 1 Length::) && step.hasValue(::UMI - Read 1 Start From Cycle::)) { fail
(::UMI - Read 1 Length must be greater than 0 if UMI - Read 1 Start From Cycle
is greater than 0.::) }; if (step.hasValue(::UMI - Read 2 Length::) &&
!step.hasValue(::UMI - Read 2 Start From Cycle::)) { fail(::UMI - Read 2 Start
From Cycle must be greater than 0 if UMI - Read 2 Length is greater than 0.::)
};
if (!step.hasValue(::UMI - Read 2 Length::) && step.hasValue(::UMI - Read 2
Start From Cycle::)) { fail(::UMI - Read 2 Length must be greater than 0 if UMI
- Read 2 Start From Cycle is greater than 0.::) }; if (!step.hasValue(::UMI -
Read 1 Length::) && step.hasValue(::UMI - Read 2 Length::)) { fail(::UMI - Read
1 Length must be greater than 0 if UMI - Read 2 Length is greater than 0.::) };
if (!step.hasValue(::UMI - Read 1 Start From Cycle::) && step.hasValue(::UMI -
Read 2 Start From Cycle::)) { fail(::UMI - Read 1 Start From Cycle must be
greater than 0 if UMI - Read 2 Start From Cycle is greater than 0.::) };
```

- Checks the Paired End and Read 2 Cycles field values.

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  - If Paired End = True and Read 2 Cycles value is 0, generates an error.
  - If Paired End = False, Read 2 Cycles must be zero, Reverse complement workflow must be false, and UMI - Read 2 Length and UMI - Read 2 Start From Cycle must be empty.

```
if (step.::Paired End::.toBoolean()) { if (step.::Read 1 Cycles:: == 0 ||
step.::Read 2 Cycles:: == 0) { fail(::Read 1 Cycles and Read 2 Cycles must be
greater than 0 if Paired End is True.::) } } else { if (step.::Read 2 Cycles::
!= 0) { fail(::Read 2 Cycles must be 0 if Paired End is False.::) }; if
(step.::Reverse Complement Workflow::.toBoolean()) { fail(::Reverse Complement
Workflow can only be enabled in a Paired End run.::) }; if (step.hasValue(::UMI
- Read 2
if (step.::Workflow Type:: == ::Dual Index:: && step.::Index Read 2:: == 0) {
  fail(::Index Read 2 must not be zero if Workflow Type is Dual Index.::)
};
if (step.::Workflow Type:: == ::No Index:: || step.::Workflow Type:: == ::Single
Index::) {
  step.::Index Read 2:: = 0
};
Length::) || step.hasValue(::UMI - Read 2 Start From Cycle::)) {
fail(::UMI - Read 2 Length and UMI - Read 2 Start From Cycle cannot be defined
if Paired End is False.::)
}
}::
```

- Checks the Flowcell Type field value.
  - If Flowcell Type value is not SP, checks that the value of both Read 1 Cycles and Read 2 Cycles is 151 or less. If value is greater than 151, it generates an error message.

```
if (input.::Flowcell Type::!=::SP:: && step.::Read 1 Cycles:: > 151) { fail
(::Read 1 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
};if (input.::Flowcell Type::!=::SP:: && step.::Read 2 Cycles:: > 151) { fail
(::Read 2 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
```

- Sets the Settings Header if UMI - Read 1 Length or UMI - Read 2 Length have value.

```
step.::Settings Header:: = step.hasValue(::UMI - Read 1 Length::) ||
step.hasValue(::UMI - Read 2 Length::) ? '[Settings]' : '';
```

- Sets the next step for samples to REMOVE.

```
nextStep = ::REMOVE::
```

- Generates the sample sheet and attaches it to the step (see *Sample Sheet Generation* section).
  - Sample sheet template used depends on the selected value of Reverse Complement Workflow.

```
if [ '{udf:Reverse Complement Workflow}' == 'true' ]; then
\templateFilename='NovaSeq_BCL2FASTQ_Reverse_Complement_Samplesheet.csv'; \elif
[ '{udf:Reverse Complement Workflow}' == 'false' ]; then
```

```
\templateFilename='NovaSeq_BCL2FASTQ_Samplesheet.csv'; \else echo 'UDF Reverse
Complement Workflow is empty or undefined.'; exit -1; fi \&&
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} \   script:driver_file_generator \     -t
/opt/gls/clarity/extensions/conf/driverfiletemplates/$templateFilename\   -o
{compoundOutputFileLuid0}.csv \     -q true \     -destLIMSID
{compoundOutputFileLuid0} \     -l{compoundOutputFileLuid1}"
```

```
UMI - Read 1 Length::) && step.hasValue(::UMI - Read 2 Length::)) {
```

```
fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 2 Length is
greater than 0.::)};if (!step.hasValue(::UMI - Read 1 Start From Cycle::) &&
step.hasValue(::UMI - Read 2 Start From Cycle::)) {fail(::UMI - Read 1 Start
From Cycle must be greater than 0 if UMI - Read 2 Start From Cycle is greater
than 0.::)};
```

- Checks the Paired End and Read 2 Cycles field values.
  - If Paired End = True and Read 2 Cycles value is 0, it generates an error.
  - If Paired End = False, Read 2 Cycles must be zero, Reverse Complement workflow must be false, and UMI - Read 2 Length and UMI - Read 2 Start From Cycle must be empty.

```
if (step.::Paired End::.toBoolean()) {if (step.::Read 1 Cycles:: == 0 ||
step.::Read 2 Cycles:: == 0) {fail(::Read 1 Cycles and Read 2 Cycles must be
greater than 0 if Paired End is True.::)}} else {if (step.::Read 2 Cycles:: !=
0) {fail(::Read 2 Cycles must be 0 if Paired End is False.::)};if
(step.::Reverse Complement Workflow::.toBoolean()) {fail(::Reverse Complement
Workflow can only be enabled in a Paired End run.::)};if (step.hasValue(::UMI -
Read 2 Length::) || step.hasValue(::UMI - Read 2 Start From Cycle::)) fail(::UMI
- Read 2 Length and UMI - Read 2 Start From Cycle cannot be defined if Paired
End is False.::)
}}::
```

- Checks the Flowcell field value.
  - If Flowcell Type value is not SP, checks that the value of both Read 1 Cycles and Read 2 Cycles is 151 or less. If value is greater than 151, it generates an error message.

```
if (input.::Flowcell Type::!=::SP:: && step.::Read 1 Cycles:: > 151) {fail
(::Read 1 Cycles must not be larger than 151 if it is not SPrime Flowcell::)}if
(input.::Flowcell Type::!=::SP:: && step.::Read 2 Cycles:: > 151) {fail(::Read 2
Cycles must not be larger than 151 if it is not SPrime Flowcell::)}
```

- Sets the Settings Header if UMI - Read 1 Length or UMI - Read 2 Length have value.

```
step.::Settings Header:: = step.hasValue(::UMI - Read 1 Length::) ||
step.hasValue(::UMI - Read 2 Length::) ? '[Settings]' : '';
```

- Sets the next step for samples to REMOVE:

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```
nextStep = ::REMOVE::
```

- Generates the sample sheet and attaches it to the step. (See the *Sample Sheet Generation* section.)
  - Sample sheet template used depends on the selected value of Reverse Complement Workflow.

```
&& if [ '{udf:Reverse Complement Workflow}' == 'true' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Reverse_Complement_Samplesheet.csv'; \
elif [ '{udf:Reverse Complement Workflow}' == 'false' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Samplesheet.csv'; \
else echo 'UDF Reverse Complement Workflow is empty or undefined.'; exit -1; fi
\
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/$templateFilename \
-o {compoundOutputFileLuid0}.csv \
-q true \
-destLIMSID {compoundOutputFileLuid0} \
-l {compoundOutputFileLuid1}"
```

Routing Script

Automatically triggered on exit of the step, this automation invokes the change Workflow script, which routes step outputs to the NovaSeq 6000 v3.0.1 workflow. It also and queues them for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0.1) step.

The default automation command line is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar
-u {username} -p {password} -i {stepURI:v2} -l {compoundOutputFileLuid2}
script:changeWorkflow \
\
--FIELD_NAME 'N/A' \
--FIELD_VALUE 'N/A' \
--WORKFLOW 'NovaSeq 6000 v3.0.1' \
--STEP 'AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0.1)' \
--INPUTS_OR_OUTPUTS 'OUTPUTS'"
```

Step UDFs/Custom Fields

There are 22 fields defined on the Dilute and Denature (NovaSeq 6000 v3.0.1) step. These fields are required for sample sheet and JSON file generation.

The fields are shown in the Clarity LIMS v5 Configuration area (Custom Fields > Master Step Fields tab). See Table 7 for Clarity LIMS v4 and Clarity LIMS v5 field configuration details.

Table 204 Table 7: Dilute and Denature (NovaSeq 6000 v3.0.1) Step UDF/Custom Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
BaseSpace Sequence Hub Configuration	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• Not Used • Run Monitoring Only • Run Monitoring and Storage
Custom Recipe Path	Single-line Text (v4) Text Dropdown (v5)	• Required: No • Read Only: No	Not applicable
Experiment Name	Single-line Text (v4) Text (v5)	• Required: Yes • Read Only: No	Not applicable
Index Read 1	Numeric (v4) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 0 • 6 • 8 • Range From: 0 to 20 • Decimal Places Displayed: 0
Index Read 2	Numeric (v4) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 0 • 6 • 8 • Range From: 0 to 20 • Decimal Places Displayed: 0
Output Folder	Single-line Text (v4) Text (v5)	• Required: Yes • Read Only: No	Not applicable
Paired End	Single-line Text (v4) Text (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• True • False

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Read 1 Cycle	Numeric (v4) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 251* • 151 • 101 • 51 • Range From: 1 to 251 • Decimal Places Displayed: 0 *Value of 251 is only supported for SP flow cell type. For all other cell types, maximum value is 151.
Read 2 Cycle	Numeric (v4) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 251* • 151 • 101 • 51 • Range From: 1 to 251 • Decimal Places Displayed: 0 *Value of 251 is only supported for SP flow cell type. For all other cell types, maximum value is 151.
Reverse Complement Workflow	Checkbox (v4) Toggle Switch (v5)	• Required: Yes • Read Only: No	• Default: Yes
Run Mode Not displayed in user interface)	Single-line Text (v4) Toggle Switch (v5)	• Required: No • Read Only: Yes • Custom Entries: No	• SP • S1 • S2 • S4
Sitting Header (not displayed in user interface)	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
UMI—Read 1 Length	Numeric (v4) Numeric (v5)	• Required: No • Custom Entries: Yes	Not applicable
UMI—Read 1 Start From Cycle	Numeric (v4) Numeric (v5)	• Required: No • Custom Entries: Yes	Not applicable
UMI—Read 2 Length	Numeric (v4) Numeric (v5)	• Required: No • Custom Entries: Yes	Not applicable
UMI—Read 2 Start From Cycle	Numeric (v4) Numeric (v5)	• Required: No • Custom Entries: Yes	Not applicable
Use Custom Index Read 1 Primer	Checkbox(v4) Toggle Switch (v5)	• Required: No • Custom Entries: No	• None set
Use Custom Read 1 Primer	Checkbox(v4) Toggle Switch (v5)	• Required: No • Custom Entries: No	• None set
Use Custom Read 2 Primer	Checkbox(v4) Toggle Switch (v5)	• Required: No • Custom Entries: No	• None set
Use Custom Recipe	Checkbox(v4) Toggle Switch (v5)	• Required: Yes • Read Only: No	• Default: No
Workflow	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	• GenerateFASTQ
Workflow Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• No Index • Single Index • Dual Index • Custom

Global UDFs/Custom Fields

Table 8 lists the global UDFs / custom fields that are configured to display on the Dilute and Denature (NovaSeq 6000 v3.0.1) step.

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Flowcell Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• SP • S1 • S2 • S4
Loading Workflow Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User Interaction

1. On the Placement screen, complete the following actions:
   a. Drag the pool into the library tube in the Placed Samples area.
   b. Scan or type the barcode of the library tube into the Library Tube field.
   c. Select Record Details.

   On exit of the Placement screen, the Check Library Tube Barcode automation checks that the library tube barcode conforms to the barcode mask [A-Za-z]{2}[0-9]{7}-[A-Za-z]{3}. An error message displays if not.
2. On the Record Details screen:
   - The Reagent Lot Tracking section tracks the NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. Select from the active lots displayed in each drop-down list.

     To make reagent lots available for selection in the Reagent Lot Tracking section, first configure and activate them. Do this step on the Reagents and Controls screen (Clarity LIMS v4.2.x) or the Configuration > Consumables screen (Clarity LIMS v5.x).

   - The fields displayed on the Record Details screen are used to set up the run and generate the sample sheet file. Some of these fields are autopopulated and some must be completed manually (see Table 9, for details).

Table 205 Table 9: Fields Displayed on Record Details Screen of Dilute and Denature (NovaSeq 6000 v3.0.1) Step

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.

Field	Value
BaseSpace Sequence Hub Configuration	Select from preset options: Not Used, Run Monitoring Only, Run Monitoring and Storage.
Workflow	Automatically populated with preset - GenerateFASTQ.
Workflow Type	Select from preset options: No Index, Single Index, Dual Index, Custom.
Index Read 1	Select from preset options: 0, 6, 8 - or type a value between 0 and 20.
Index Read 2	Select from preset options: 0, 6, 8 - or type a value between 0 and 20.
Paired End	Select from preset options: True or False.
Read 1 Cycles	Select from preset options: 251, 151, 101, 51 - or type a value between 1 and 251. Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Read 2 Cycles	Select from preset options: 251, 151, 101, 51 - or type a value between 1 and 251. Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Use Custom Read 1 Primer	Select if applicable.
Use Custom Read 2 Primer	Select if applicable.
Use Custom Index Read 1 Primer	Select if applicable.
Reverse Complement Workflow	Select Yes (by default) for v1.5 reagents in NovaSeq Control Software v1.7 and onwards. Select No for v1 reagents.
Use Custom Recipe	Select if applicable.
Custom Recipe Path	If the Use Custom Recipe option was selected, enter the path to the custom recipe file to be used.
UMI - Read 1 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 1. Leave blank if not applicable.
UMI - Read 2 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 2. Leave blank if not applicable.

Field	Value
UMI - Read 1 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.
UMI - Read 2 Start From Cycle	Enter the cycle number that Read 2 starts from. Leave blank if not applicable.
Output Folder	Enters the network path for sequencing run folder. For example: \\networkshare\run_data

3. On the Record Details screen, select Validate Run Setup and Generate Sample Sheet.
   This selection triggers the automation script, which does the following:
   a. Validates the information provided to set up the run.
   b. Generates the sample sheet file and attaches it to the placeholder in the Files area of the Record Details screen.
   c. Sets the value of the next step to Remove from workflow. The Routing Script automation expects this value and requires it to successfully advance samples to the next step.
4. Select Next Steps.
   On the Assign Next Steps screen, the Next Step field for all samples is prepopulated with Remove from workflow.

   Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.

5. Select Finish Step.
   On exit from the step:
   - The Routing Script automation is triggered and samples are routed to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0.1) protocol.
   - In Lab View, the pool of samples is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0.1) step.

At this point in the workflow, the user interaction ends. The integration automatically starts the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0.1) step and data from the run is parsed back into Clarity LIMS.

Protocol 3: NovaSeq Xp (NovaSeq 6000 v3.0.1)

Samples are routed to this protocol if their Loading Workflow Type value is set to NovaSeq Xp.

Samples are pooled and added to lanes on the NovaSeq flow cell type selected in the Define Run Format (NovaSeq 6000 v3.0.1) step. At the end of this protocol, the flow cell is sent to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0.1) protocol.

This protocol contains three steps:

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- Step 1: Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.0.1)
- Step 2: Dilute, Denature, and ExAmp (NovaSeq 6000 v3.0.1)
- Step 3: Load to Flowcell (NovaSeq 6000 v3.0.1)

Step 1: Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.0.1)

In this step, manually place libraries into a pool.

Only create one pool per step.

Step input: NTP (normalized libraries)

Step output: Bulk pool

The following automations are configured on the step:

- Calculate Volumes
- Set Next Step
- Validate Inputs Flowcell Type and Single Pool
- Validate Unique Indexes (Not used. This functionality is handled by the core Clarity LIMS configuration for pooling.)

These automations are described in the order in which they are triggered at run time.

Validate Inputs Flowcell Type and Single Pool

Automatically triggered on exit of the Pooling screen, this automation does the following actions:

- Checks that all samples in the pool have the same Flowcell Type assigned to them.
- Checks that only one pool has been created.
- Changes the value of error and logging messages to reference the type of pool passed as bulk pool.

```
/opt/gls/clarity/bin/java \-jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar \script:validate_flowcell_for_input_pools \
  -i {stepURI:v2} \
  -u {username} \
  -p {password} \
  -l {compoundOutputFileLuid1} \
  -validateSingleOutput true \
  -poolType bulk"
```

Calculate Volumes

Automatically triggered when the Calculate Volumes button is selected on the Record Details screen, this automation does the following actions:

- Calculates the number of samples in the pool:

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: + 1
```

- Sets the value of the Bulk Pool Volume (µl) field, based on the selected Flowcell Type:

```
if ( input.::Flowcell Type:: == ::SP:: ) {
  step.::Bulk Pool Volume (µl):: = step.::Number of Lanes to Sequence:: * 18 +
30
} ;
if ( input.::Flowcell Type:: == ::S1:: ) {
  step.::Bulk Pool Volume (µl):: = step.::Number of Lanes to Sequence:: * 18 +
30
} ;
if ( input.::Flowcell Type:: == ::S2:: ) {
  step.::Bulk Pool Volume (µl):: = step.::Number of Lanes to Sequence:: * 22 +
30
} ;
if ( input.::Flowcell Type:: == ::S4:: ) {
  step.::Bulk Pool Volume (µl):: = step.::Number of Lanes to Sequence:: * 30 +
30
} ;
```

- Calculates the Per Sample Volume (µl) to be added to the pool:

```
input.::Per Sample Volume (µl)::= (((input.::Final Loading Concentration (pM)::*
5/ 1000) / input.::Normalized Molarity (nM)::) * step.::Bulk Pool Volume (µl)::)
/ step.::Number of Samples in Pool::
```

> To ensure accurate pipetting of each sample in a pool for sequencing, the Per Sample Volume (µl) value must be equal to or higher than the Minimum Per Sample Volume (µl). The default value, set at 5, may be edited.

Assuming the default Minimum Per Sample Volume (µl) value of 5, for a given batch:

1. If the smallest Per Sample Volume (µl) value is less than 5, Clarity LIMS automatically assigns a value of 5 to the sample's Adjusted Per Sample Volume (µl) field.
2. Clarity LIMS then adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

- Calculates the Total Sample Volume (µl) field value:

```
step.::Total Sample Volume (µl):: = step.::Total Sample Volume (µl):: +
input.::Adjusted Per Sample Volume (µl)::
```

- If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) field value:

```
if (step.::Total Sample Volume (µl):: >= step.::Bulk Pool Volume (µl)::)
{output.::RSB Volume (µl):: = 0} else {output.::RSB Volume (µl):: = step.::Bulk
Pool Volume (µl):: - step.::Total Sample Volume (µl)::} ;
```


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- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs:

```
output.::Flowcell Type::= input.::Flowcell Type::;output.::Loading Workflow
Type::= input.::Loading Workflow Type::;
```

- Uses the `NovaSeq_Xp_Bulk_Pool.csv` and `NovaSeq_Xp_Bulk_Pool2.csv` template files to generate a single CSV file containing information about the bulk pool and the samples it contains. The generated file is available for download on the Step Setup screen of the following step: Dilute, Denature, and ExAmp (NovaSeq 6000 v3.0.1).

```
&& /opt/gls/clarity/bin/java \-jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar \
  -i {stepURI:v2} \
  -u {username} \
  -p {password} \
  -l {compoundOutputFileLuid1} \ script:driver_file_generator \
  -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_Bulk_
Pool.csv \
  -o {compoundOutputFileLuid0}.csv \ script:driver_file_generator \
  -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_Bulk_
Pool2.csv \
  -o append.csv \
&& cat append.csv >> {compoundOutputFileLuid0}.csv"
```

Set Next Step

Automatically triggered on exit of the Record Details screen, this automation:

- Copies the Flowcell Type values from the step inputs to the step outputs.
- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ; nextStep = ::ADVANCE::
```

Step UDFs/Custom Fields

There are five fields defined on the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.0.1) step.

These fields are shown in the Clarity LIMS v5 Configuration area (Custom Fields > Master Step Fields tab).

See the following table for Clarity LIMS v4 and Clarity LIMS v5 field configuration details.

Table 206 Table 10: Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.0.1) Step UDF/Custom Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Bulk Pool Volume (µl) (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2
Number of Lanes to Sequence	Numeric	• Required: Yes • Read Only: No	• Decimal Places Displayed: 0
Total Sample Volume (µl) (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Default: 0 • Decimal Places Displayed: 0
Minimum Per Sample Volume (µl)	Numeric	• Required: Yes • Read Only: No	• Default: 5 • Decimal Places Displayed: 2
Number of Samples in Pool (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Default: 0 • Decimal Places Displayed: 0

Global UDFs/Custom Fields

Table 11 lists the global UDFs/custom fields that are configured to display on the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.0.1) step.

Table 207 Table 11: Global UDF/Custom Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Configured on Analyte in Clarity LIMS v4 and Derived Sample in Clarity LIMS v5:			
RSB Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Flowcell Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• SP • S1 • S2 • S4
Loading Workflow Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User Interaction

1. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucket and select View Ice Bucket.
2. On the Ice Bucket screen, select Begin Work.
3. On the Pooling screen:
   a. Drag samples into the Pool Creator to create a pool.
   b. Name the pool or accept the default name (Pool #1).
   c. Select Record Details.

   On exit from the Pooling screen, the Validate Inputs Flowcell Type and Single Pool automation is triggered. The automation checks that all samples in the pool have been assigned the same Flowcell Type and only one pool has been created.
4. On the Record Details screen, the Step Details area contains two required fields:
   - Number of Lanes to Sequence—The value entered in this field is used in volume calculations, to make sure that volumes are sufficient for the number of times the pool is sequenced.
   - Minimum Per Sample Volume (µl)—In many applications, and particularly where robotics are used, a minimum sample volume is required. The value in this field is used to calculate how much of each sample is included in the pool. The field is prepopulated with the configured default value, 5 µl, but may be edited if necessary.

   In the Sample Details table, select the pool icon to view details on the pool composition.
5. Select Calculate Volumes. This selection triggers the Calculate Volumes automation, which performs volume calculations based on the selected Flowcell Type:
   - Calculates Bulk Pool Volume (µl) value.
   - Calculates the Per Sample Volume (µl) value to be added to the pool.

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- Calculates the Total Sample Volume (µl) value.
- If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) value.
- Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
- Generates the Calculation File (*csv) and attaches it to the step. This file contains volume information about the samples and RSB buffer to add to the pool. Select the file to download it, then open it in Excel.

6. Select Next Steps. This selection triggers the Set Next Step automation. This automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol: Dilute, Denature, and ExAmp (NovaSeq 6000 v3.0.1).
   On the Assign Next Steps screen, the next step is already set to Dilute, Denature, and ExAmp (NovaSeq 6000 v3.0.1).
7. Select Finish Step. At the end of this step, the pool of samples automatically advances to the Dilute, Denature, and ExAmp (NovaSeq 6000 v3.0.1) step.

Step 2: Dilute, Denature and ExAmp (NovaSeq 6000 v3.0.1)

In this step, pooled samples are denatured and diluted by the addition of DPX, NaOH, Tris-HCl, and RSB. Manually create working pools based on the number of lanes you want to sequence.

Step input: Bulk pool

Step output: Working pool - variable number, choose how many working pools to create per bulk pool

The following automations are configured on the step:

- Calculate Volumes
- Set Next Step
- Validate Inputs Flowcell Type
- Dilute, Denature, and ExAmp (NovaSeq 6000 v3.0.1) automation configuration - Clarity LIMS v5

These automations are described in the order in which they are triggered at run time.

Validate Inputs Flowcell Type

Automatically triggered at the beginning of the step, this automation invokes the validate_flowcell_for_input_pools script. This script does the following:

- Checks the inputs to the step and validates that the Flowcell Type field has been set to a valid value (SP, S1, S2, or S4) and that all inputs have the same value for this field.
- Checks the container type selected matches the value in the Flowcell Type field.
- Validates that the number of outputs matches the number of lanes on the selected flow cell type. If validation fails, an error message reveals that the number of working pools does not match the number of lanes available on the flow cell.

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```
bash -l -c "/opt/gls/clarity/bin/java \
-jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar
\script:validate_flowcell_for_input_pools \
  -i {stepURI:v2} \
  -u {username} \
  -p {password} \
  -l {compoundOutputFileLuid1}"
```

Calculate Volumes

Automatically triggered on entry to of the Record Details screen, this automation sets the value of the following fields, based on the Flowcell Type:

- Bp Aliquot Volume (µl)
- NaOH Volume (µl)
- Tris-HCl Volume (µl)
- DPX1 Volume (µl)
- DPX2 Volume (µl)
- DPX3 Volume (µl)

```
'if ( input.::Flowcell Type:: == ::SP:: ) {
  output.::bp Aliquot Volume (µl):: = 18 ;
  output.::NaOH Volume (µl):: = 4.50 ;
  output.::Tris-HCl Volume (µl):: = 4.50 ;
  step.::DPX1 Volume (µl):: = 126 ;
  step.::DPX2 Volume (µl):: = 18 ;
  step.::DPX3 Volume (µl):: = 66 ;
  output.::Mastermix per Lane (µl):: = 63};
if (input.::Flowcell Type:: == ::S1::) {
  output.::bp Aliquot Volume (µl):: = 18;
  output.::NaOH Volume (µl):: = 4.50;
  output.::Tris-HCl Volume (µl):: = 4.50;
  step.::DPX1 Volume (µl):: = 126;
  step.::DPX2 Volume (µl):: = 18;
  step.::DPX3 Volume (µl):: = 66;
  output.::Mastermix per Lane (µl):: = 63};
if (input.::Flowcell Type:: == ::S2::) {
  output.::bp Aliquot Volume (µl):: = 22;
  output.::NaOH Volume (µl):: = 5.50;
  output.::Tris-HCl Volume (µl):: = 5.50;
```

```
  step.::DPX1 Volume (µl):: = 126;
  step.::DPX2 Volume (µl):: = 18;
  step.::DPX3 Volume (µl):: = 66;
  output.::Mastermix per Lane (µl):: = 77};
if (input.::Flowcell Type:: == ::S4::) {
  output.::bp Aliquot Volume (µl):: = 30;
  output.::NaOH Volume (µl):: = 7.50;
  output.::Tris-HCl Volume (µl):: = 7.50;
  step.::DPX1 Volume (µl):: = 315;
  step.::DPX2 Volume (µl):: = 45;
  step.::DPX3 Volume (µl):: = 165;
  output.::Mastermix per Lane (µl):: = 105
};
```

The automation also does the following actions:

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs:

```
output.::Flowcell Type::= input.::Flowcell Type::;
output.::Loading Workflow Type::= input.::Loading Workflow Type::;
```

- Uses the `NovaSeq_Xp_Working_Pool.csv` and `NovaSeq_Xp_Working_Pool2.csv` template files to generate a single CSV file. The file contains information about the DPX volume and the volume of bp Aliquot, Mastermix, NaOH, and Tris-HCl to add per working pool. The generated file is available for download on the Step Setup screen of the following step: Load to Flowcell (NovaSeq 6000 v3.0.1).

```
and /opt/gls/clarity/bin/java \
-jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/DriverFileGenerator.jar \
 -i {stepURI:v2} \
 -u {username} \
 -p {password} \
 -l {compoundOutputFileLuid1} \script:driver_file_generator \
 -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_Working_
Pool.csv \
 -o {compoundOutputFileLuid0}.csv \script:driver_file_generator \
 -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_Working_
Pool2.csv \
 -o append.csv \
&& cat append.csv >> {compoundOutputFileLuid0}.csv"
```

Set Next Step

Automatically triggered on exit of the Record Details screen, this automation does the following actions:

- Copies the Flowcell Type values from the step inputs to the step outputs.
- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ; nextStep = ::ADVANCE::
```

Step UDFs/custom fields

There are three fields defined on the Dilute, Denature, and ExAmp (NovaSeq 6000 v3.0) step. These field values are set by a script and are not editable while running the step.

The fields are shown in the Clarity LIMS v5.x Configuration area (Custom Fields > Master Step Fields tab).

Table 12 lists Clarity LIMS v4 and Clarity LIMS v5 field configuration details.

Table 208 Table 12: Dilute, Denature, and ExAmp (NovaSeq v3.0.1) Step UDF/Custom Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
DPX1 Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0
DPX2 Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0
DPX3 Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0

Global UDFs/Custom Fields

Table 209 Table 13: Global UDFs/Custom Fields on the Dilute, Denature, and ExAmp (NovaSeq 6000 v3.0.1) Step

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Configured on Analyte in Clarity LIMS v4 and Derived Sample in Clarity LIMS v5:			
Bp Aliquot Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Flowcell Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• SP • S1 • S2 • S4
Loading Workflow Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
Mastermix per Lane (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0
NaOH Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Tris-HCl Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2

User Interaction

1. On the Queue screen, add the pool to the Ice Bucket and select View Ice Bucket.
2. On the Ice Bucket screen, (optionally) set the number of derivatives to create. Place them into the flow cell lanes and select Begin Work.
   On entry to the Record Details screen, the Calculate Volumes automation is triggered. This automation sets the following values based on the selected Flowcell Type (calculation details are provided in the previous *Calculate Volumes* section):
   - Bp Aliquot Volume (µl)
   - NaOH Volume (µl)
   - Tris-HCl Volume (µl)
   - DPX1 Volume (µl)
   - DPX2 Volume (µl)
   - DPX3 Volume (µl)
   - Mastermix per Lane (µl)

   Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.

Generates the Calculation File (*csv) and attaches it to the step. This file contains information about the DPX Mastermix volume and the volume of Mastermix, NaOH, and Tris-HCl to add per working pool. Select the file to download it, then open it in Excel.

3. On the Record Details screen, the Reagent Lot Tracking section tracks the DPX1, DPX2, DPX3, NaOH, RSB, and Tris-HCl reagent lots. Select from the active lots displayed in each drop-down list.

   > To make reagent lots available for selection in the Reagent Lot Tracking section, first configure and activate them. Do this step on the Reagents and Controls screen (Clarity LIMS v4) or the Configuration > Consumables screen (Clarity LIMS v5).

   In the Step Details area, the DPX1, DPX2, and DPX3 reagent volume values are already populated. (These values are set by a script and are not editable.)
4. In the Sample Details table, select the pool icon to view details on the working pool composition.
   - The bp Aliquot, Mastermix per lane, NaOH, and Tris-HCl volume values for each working pool are already populated. (These values are set by a script and are not editable.)
   - The working pool number is appended to the bulk pool name. This feature allows you to quickly identify which working pools are derived from the same bulk pool.
5. In the Files area, select the Calculation File (*csv) file to download it and then open it in Excel to view information about the DPX Mastermix volume, and the volume of Mastermix, NaOH. and Tris-HCl to add per working pool.
6. Select Next Steps to trigger the Set Next Step automation (described previously).
   On the Assign Next Steps screen, the next step is already set to Load to Flowcell (NovaSeq 6000 v3.0.1).
7. Select Finish Step.

Step 3: Load to Flowcell (NovaSeq 6000 v3.0.1)

In this step, scan the flow cell barcode into Clarity LIMS, then manually place the working pools into the lanes of the flow cell that are used in the NovaSeq run. In addition, this step validates the run setup information and generates the sample sheet file.

Step input: Working pool

Step output: Flow cell (output containers: SP, S1, and S2 with 2 lanes, and S4 with 4 lanes)

The following automations are configured on the step:

- Validate Run Setup and Generate Sample Sheet
- Validate Flowcell Barcode
- Validate Inputs and Selected Container
- Load to Flowcell (NovaSeq 6000 v3.0.1) automation configuration - Clarity LIMS v5

These automations are described in the order in which they are triggered at run time.

Validate Inputs and Selected Container

Automatically triggered at the beginning of the step, this automation invokes the validate_flowcell_for_input_pools and validateSelectedContainer scripts. These scripts validate the step inputs and the container selected, as follows.

- Check that the Flowcell Type field has been set to a valid value (SP, S1, S2, or S4), and that all inputs have the same value.
- Check that the number of outputs matches the number of lanes on the selected flow cell type. If validation fails, an error message says that the number of working pools does not match the number of lanes available on the flow cell.
  The following table defines the number of samples allowed:

Flow Cell Type	Number of Working Pools
SP	2
S1	2
S2	2
S4	4

- Check that the container type selected on entry to the Placement screen matches the value in the Flowcell Type field. If validation fails, an error message displays.

```
bash -l -c "/opt/gls/clarity/bin/java \
-jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar
\script:validate_flowcell_for_input_pools \
  -i {stepURI:v2} \
  -u {username} \
  -p {password} \
  -l {compoundOutputFileLuid1} \
  -validateSelectedContainer true"
```

Validate Flowcell Barcode

Automatically triggered on exit of the Placement screen, this automation:

Validates the flow cell barcode scanned into Clarity LIMS, using the following logic:

```
if (input.::Flowcell Type:: == ::SP:: && !output.container.name.matches( ::[A-
Za-z0-9]{5}DRXX:: ) ) { \
  fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: ) \
} ; \
if (input.::Flowcell Type:: == ::S1:: && !output.container.name.matches( ::[A-
Za-z0-9]{5}DRXX:: ) ) { \
  fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: ) \
} ; \
```

```
if (input.::Flowcell Type:: == ::S2:: && !output.container.name.matches( ::[A-Za-z0-9]{5}DMXX:: ) ) { \
  fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: ) \
} ; \
if (input.::Flowcell Type:: == ::S4:: && !output.container.name.matches( ::[A-Za-z0-9]{5}DSXX:: ) ) { \
  fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: ) \
} ;
```

Copies the Flowcell Type and Loading Workflow Type field values from step inputs to outputs:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ; \
output.::Loading Workflow Type:: = input.::Loading Workflow Type::'
```

Validate Run Setup and Generate Sample Sheet

Automatically triggered when a button on the Record Details screen is selected, this automation does the following actions:

- Copies the Flowcell Type from the step input to the Run Mode field (hidden):

```
step.::Run Mode:: = input.::Flowcell Type::
```

- Validates the parameters entered on the Record Details screen, which is used to set up the run and generate the sample sheet file. (See*Respond to Validation/Recipe Request Call from Instrument* on page 768 section.)
  - The Experiment Name field may only contain alphanumeric, dash, or underscore characters. Spaces are not permitted.
  - When Workflow Type = No Index, both Index Read 1 and Index Read 2 must be zero.
  - When Workflow Type = Single Index, Index Read 1 must be greater than zero and Index Read 2 must be zero.
  - When Workflow Type = Dual Index, both Index Read 1 and Index Read 2 must be greater than zero.

```
if(!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) { \
  fail(::Experiment Name contains prohibited characters. Allowed characters are: a-z, A-Z, 0-9, -, and _::) \
}; \
if(step.::Workflow Type::== ::No Index::) { \
  if(step.::Index Read 1::!= 0) { \
    fail(::Index Read 1 must be 0 if the Workflow Type is No Index.::) \
  } \
} else { \
  if(step.::Index Read 1::== 0) { \
```


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```
    fail(::Index Read 1 must be greater than 0 if the Workflow Type is ::+
step.::Workflow Type::+ ::.::) \
  } \
};
```

- Validate UMI Settings:
  - If UMI - Read 1 Length has value, then UMI - Read 1 Start From Cycle must have value.
  - If UMI - Read 1 Length has no value, then UMI - Read 1 Start From Cycle must not have value.
  - If UMI - Read 2 Length has value, then UMI - Read 2 Start From Cycle must have value.
  - If UMI - Read 2 Length has no value, then UMI - Read 2 Start From Cycle must not have value.
  - If UMI - Read 1 Length has not value, then UMI - Read 2 Length must not have value.
  - If UMI - Read 1 Start From Cycle has no value, then UMI - Read 2 Start From Cycle must not have value.

```
if (step.hasValue(::UMI - Read 1 Length::) && !step.hasValue(::UMI - Read 1
Start From Cycle::)) {
fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 1
Length is greater than 0.::)
};
if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 1
Start From Cycle::)) {
fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 1 Start From
Cycle is greater than 0.::)
};
if (step.hasValue(::UMI - Read 2 Length::) && !step.hasValue(::UMI - Read 2
Start From Cycle::)) {
fail(::UMI - Read 2 Start From Cycle must be greater than 0 if UMI - Read 2
Length is greater than 0.::)
};
if (!step.hasValue(::UMI - Read 2 Length::) && step.hasValue(::UMI - Read 2
Start From Cycle::)) {
fail(::UMI - Read 2 Length must be greater than 0 if UMI - Read 2 Start From
Cycle is greater than 0.::)
};
if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 2
Length::)) {
fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 2 Length is
greater than 0.::)
};
if (!step.hasValue(::UMI - Read 1 Start From Cycle::) && step.hasValue(::UMI -
Read 2 Start From Cycle::)) {
```


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```
fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 2
Start From Cycle is greater than 0.::)
};
```

- Checks the Paired End and Read 2 Cycles field values.
  - If Paired End = True and Read 2 Cycles value is 0, an error message is generated.
  - If Paired End = False, Read 2 Cycles must be zero and the Reverse Complement workflow must be false. UMI - Read 2 Length and UMI - Read 2 Start From Cycle must be empty.

```
if (step.::Paired End::.toBoolean()) {
if (step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) {
fail(::Read 1 Cycles and Read 2 Cycles must be greater than 0 if Paired End is
True.::)
}
} else {
if (step.::Read 2 Cycles:: != 0) {
fail(::Read 2 Cycles must be 0 if Paired End is False.::)
};
if (step.::Reverse Complement Workflow::.toBoolean()) {
fail(::Reverse Complement Workflow can only be enabled in a Paired End run.::)
};
if (step.hasValue(::UMI - Read 2 Length::) || step.hasValue(::UMI - Read 2 Start
From Cycle::)) {
fail(::UMI - Read 2 Length and UMI - Read 2 Start From Cycle cannot be defined
if Paired End is False.::)
}
}::
```

- Checks the Flowcell field value.
  - If Flowcell Type value is not SP, checks that the value of both Read 1 Cycles and Read 2 Cycles is 151 or less. If value is greater than 151, an error message is generated.

```
if (input.::Flowcell Type::!=::SP:: && step.::Read 1 Cycles:: > 151) {
fail(::Read 1 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
}
if (input.::Flowcell Type::!=::SP:: && step.::Read 2 Cycles:: > 151) {
fail(::Read 2 Cycles must not be larger than 151 if it is not SPrime Flowcell::)
}
```

- Sets the Settings Header if UMI - Read 1 Length or UMI - Read 2 Length have value.

```
step.::Settings Header:: = step.hasValue(::UMI - Read 1 Length::) ||
step.hasValue(::UMI - Read 2 Length::) ? '[Settings]' : '';
```


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- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol - AUTOMATED - Run (NovaSeq 6000) v3.0.

```
nextStep = ::ADVANCE::
```

- Generates the sample sheet and attaches it to the step. (See the *Sample Sheet Generation* section.)
- Sample sheet template used depends on the selected value for the Reverse Complete Workflow.

```
&& if [ '{udf:Reverse Complement Workflow}' == 'true' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Reverse_Complement_Samplesheet.csv'; \
elif [ '{udf:Reverse Complement Workflow}' == 'false' ]; then \
templateFilename='NovaSeq_BCL2FASTQ_Samplesheet.csv'; \
else echo 'UDF Reverse Complement Workflow is empty or undefined.'; exit -1; fi
\
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/$templateFilename \
-o {compoundOutputFileLuid0}.csv \
-q true \
-destLIMSID {compoundOutputFileLuid0} \
-l {compoundOutputFileLuid1}"
```

Step UDFs/Custom Fields

There are 23 fields defined on the Load to Flowcell (NovaSeq 6000 v3.0.1) step.

These fields are shown in the Clarity LIMS v5 Configuration area (Custom Fields > Master Step Fields tab). See Table 14 for Clarity LIMS v4 and Clarity LIMS v5 field configuration details.

Field Name	Field Type	Field Contraints/Options	Preset Values/Additional Options and Dropdown Items
BaseSpace Sequence Hub Configuration	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• Not Used • Run Monitoring Only • Run Monitoring and Storage
Custom Recipe Path	Single-line Text (v4) Text (v5)	• Required: No • Read Only: No	Not applicable

Field Name	Field Type	Field Contraints/Options	Preset Values/Additional Options and Dropdown Items
Experiment Name	Single-line Text (v4) Text (v5)	• Required: Yes • Read Only: No	Not applicable
Index Read 1	Numeric (v4) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 0 • 6 • 8 • Range From: 0 to 20 • Decimal Places Displayed: 0
Index Read 2	Numeric (v4) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 0 • 6 • 8 • Range From: 0 to 20 • Decimal Places Displayed: 0
Library Tube Barcode	Single-line Text (v4) Text (v5)	• Required: Yes • Read Only: No	Not applicable
Paired Ends	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• True • False

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Field Name	Field Type	Field Contraints/Options	Preset Values/Additional Options and Dropdown Items
Read 1 Cycles	Numeric (v4) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• *251 • 151 • 101 • 51 • Range From: 1 to 251 • Decimal Places Displayed: 0 • *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151
Read 2 Cycles	Numeric (v4) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• *251 • 151 • 101 • 51 • Range From: 1 to 251 • Decimal Places Displayed: 0 • *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151
Reverse Complement Workflow	Checkbox (v4) Toggle Switch (v5)	• Required: Yes • Read Only: No	• Default: Yes

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Field Name	Field Type	Field Contraints/Options	Preset Values/Additional Options and Dropdown Items
Run Mode (not displayed in user interface)	Single-line Text (v4) Text Dropdown (v5)	• Required: No • Read Only: Yes • Custom Entries: No	• SP • S1 • S2 • S4
Setting Header (not displayed in user interface)	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable
UMI—Read 1 Length	Numeric (v4) Numeric (v5)	• Required: No • Read Only: Yes	Not applicable
UMI—Read 1 Start From Cycle	Numeric (v4) Numeric (v5)	• Required: No • Read Only: Yes	Not applicable
UMI—Read 2 Length	Numeric (v4) Numeric (v5)	• Required: No • Read Only: Yes	Not applicable
UMI—Read 2 Start From Cycle	Numeric (v4) Numeric (v5)	• Required: No • Read Only: Yes	Not applicable
Use Custom Index Read 1 Primer	Checkbox (v4) Toggle Switch (v5)	• Required: No • Read Only: No	• None Set
Use Custom Read 1 Primer	Checkbox (v4) Toggle Switch (v5)	• Required: No • Read Only: No	• None Set
Use Custom Read 2 Primer	Checkbox (v4) Toggle Switch (v5)	• Required: No • Read Only: No	• None Set
Use Custom Recipe	Checkbox (v4) Toggle Switch (v5)	• Required: Yes • Read Only: No	Default: No
Workflow	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	• GenerateFASTQ
Workflow Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• No Index • Single Index • Dual Index • Custom

Global UDFs/Custom Fields

Table 15 lists the global UDFs/custom fields that are configured to display on the Load to Flowcell (NovaSeq 6000 v3.0.1) step.

Table 210 Table 15: Global UDF/Custom Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Configured on Analyte in Clarity LIMS v4 and Derived Sample in Clarity LIMS v5:			
Flowcell Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• SP • S1 • S2 • S4
Loading Workflow Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User Interaction

1. On the Ice Bucket screen:
   a. Select the appropriate flow cell type from the Destination Container drop-down list in the Container Options panel.
   b. Select Begin Work.
2. On the Placement screen:
   a. Drag the pool (or pools) from the left of the screen over into the Placed Samples area on the right.
   b. Scan or types the barcode of the flow cell into the flow cell field.
   c. Select Record Details.

   On exit of the Placement screen, the Validate Flowcell Barcode automation validates the container barcode (described previously).
3. The fields displayed on the Record Details screen are used to set up the run and generate the sample sheet file. Some of these fields are autopopulated. Manually complete fields as needed (see Table 16 for more details).

Table 211 Table 16: Fields Displayed on Record Details Screen of Load to Flowcell (NovaSeq 6000 v3.0.1) Step

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.

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Field	Value
BaseSpace Sequence Hub Configuration	Select from preset options: Not Used, Run Monitoring Only, Run Monitoring and Storage.
Workflow	Automatically populated with preset—GenerateFASTQ.
Workflow Type	Select from preset options: No Index, Single Index, Dual Index, Custom.
Index Read 1	Select from preset options: 0, 6, 8—or type a value between 0 and 20.
Index Read 2	Select from preset options: 0, 6, 8—or type a value between 0 and 20.
Paired End	Select from preset options: True or False.
Read 1 Cycles	Select from preset options: 251, 151, 101, 51—or type a value between 1 and 251. Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Read 2 Cycles	Select from preset options: 251, 151, 101, 51—or type a value between 1 and 251. Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Use Custom Read 1 Primer	Select if applicable.
Use Custom Read 2 Primer	Select if applicable.
Use Custom Index Read 1 Primer	Select if applicable.
Reverse Complement Workflow	Select Yes (by default) for v1.5 reagents in NovaSeq Control Software v1.7 and onwards. Select No for v1 reagents.
Use Custom Recipe	Select if applicable.
Custom Recipe Path	If Use Custom Recipe selected, enter the path to the custom recipe file to be used.
UMI - Read 1 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 1. Leave blank if not applicable.
UMI - Read 2 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 2. Leave blank if not applicable.

Field	Value
UMI - Read 1 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.
UMI - Read 2 Start From Cycle	Enter the cycle number that Read 2 starts from. Leave blank if not applicable.
Output Folder	Enter the network path for sequencing run folder. For example: \\networkshare\run_data
Library Tube Barcode	Scan the library tube barcode.

4. On the Record Details screen, select Validate Run Setup and Generate Sample Sheet.
   This selection triggers the automation script, which does the following actions:
   - Validates the information provided to set up the run.
   - Generates the sample sheet file and attaches it to the placeholder in the Files area of the Record Details screen.
5. Select Next Steps.
   On the Assign Next Steps screen, the Next Step field for all samples is prepopulated with Mark protocol as complete.
6. Select Finish Step.
   In Lab View, the pooled samples are queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0.1) step.

At this point in the workflow, the user interaction ends. The integration automatically starts the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0.1) step and data from the run is parsed back into Clarity LIMS.

Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0.1)

This final protocol contains one fully automated step, AUTOMATED- NovaSeq Run (NovaSeq 6000 v3.0.1).

This step is fully automated. Do not add samples to the Ice Bucket or start the step manually.

Step 1: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0.1)

Step input: Library tube from NovaSeq Standard or flow cell from NovaSeq Xp protocol

Step output: Result file/measurement

Do not start this step. This step is fully automated, and the sequencing service does not update samples correctly if they have been manually started.

In this step, pooled samples are sequenced on the NovaSeq instrument and the run metrics are recorded in Clarity LIMS.

Automated - NovaSeq Run (NovaSeq 600 v3.0.1) step contains two automations:

- Set Next Steps
- Update Lane Number

The Set Next Steps automation is the only automation configured on this step. By default, this automation is disabled (set to Not Used) and the Sequencer API is used to set the next step for samples.

AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0.1) automation configuration - Clarity LIMS v5.

Set Next Steps

If necessary, override the default next step behavior used by the Sequencer API. To do this override, follow these steps:

1. Enable the automation and use it to set custom next step behavior and generate a log file. By default, the automation sets the next step to ADVANCE and the samples complete the protocol.
2. Configure the automation to be triggered automatically on exit of the Record Details screen.

Update Lane Number

This automation is triggered automatically upon entry to the Record Details screen.

Do not disable or modify this automation to ensure the Lane Number displays properly.

Step UDFs/Custom Fields

Table 17 shows how the run parameters parsed from the `RunParameter.xml` file map to step UDFs/custom fields configured on the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0.1) step in Clarity LIMS.

Table 212 Table 17: Step UDFs/Custom Fields

Step UDF/Custom Field Name
Current Cycle
Current Read
Firmware Version
Flow Cell Expiration Date
Flow Cell ID
Flow Cell Lot Number
Flow Cell Mode

Step UDF/Custom Field Name
Flow Cell Part Number
Flow Cell Side
Instrument Control Software Version
Instrument ID
Instrument Type
Lane Counter
Loading Workflow Type
Output Folder
RTA Version
Run ID
Run Status
Sequencing Log

Global UDFs/Custom Fields

The following global UDFs/custom fields are used to capture the run metrics in Clarity LIMS.

- % Aligned R1
- % Aligned R2
- % Bases >=Q30 R1
- % Bases >=Q30 R2
- % Error Rate R1
- % Error Rate R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- %PF R1
- %PF R2
- Cluster Density (K/mm^2) R1
- Cluster Density (K/mm^2) R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2

- Reads PF (M) R1
- Reads PF (M) R2
- Yield PF (Gb) R1
- Yield PF (Gb) R2

Note the following information:

- Values are aggregated across all lanes. Some values—for example Yield PF (Gb) R1—are summed, while others are averaged.
- The names listed previously are the default UDF/global field names installed with the NovaSeq Integration v3.2.2 configuration provided in the Preconfigured Workflow Package (PCWP) v1.2.2 or later.
- All UDFs/global fields are configured on the Container entity.
- All field names are configurable via the Clarity LIMS Configuration > Custom Fields screen (Global Fields tab).
- If the field names in Clarity LIMS are changed, also change them through the `run.metricUdfNames` properties file in the `application.yml` file for the Sequencer API (see *application.yml Properties* on page 773). All fields are configured to be visible on the Record Details screen of the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0.1) step, in the Sample Details table.
  - As these UDFs/fields are configured on the Container entity, they are visible on each input. However, they should not be confused with per-lane metrics.
  - All run metrics tracked in Clarity LIMS are overall metrics for the run. There are no per-lane metrics provided by NovaSeq Control Software currently.

How the Sequencer API Integration Works

Sample Sheet Generation

The sample sheet is generated on the step before the run – Dilute and Denature (NovaSeq 6000 v3.0.1) in the NovaSeq Standard protocol or Load to Flowcell (NovaSeq 6000 v3.0.1) in the NovaSeq Xp protocol. This step is where samples are placed on the library tube or flow cell that is loaded in the NovaSeq instrument.

In the default configuration, the *Validate Run Setup and Generate Sample Sheet* on page 754 automation generates one CSV format sample sheet file for use with bcl2fastq v2.20 downstream analysis.

How it Works

The sample sheet is uploaded to the NVCS via the /Illumina/Sequencer/v2/sequencing-run/files endpoint to the Sequencer API. The files endpoint allows for a file to be downloaded from Clarity LIMS using OAuth (instead of Basic Authentication), which is required for NovaSeq.

The run recipe response sets the sample sheet URL to the link to download the file from this endpoint and sets the sampleSheetRequiresOAuth value to true.

Sample Sheet Contents

The sample sheet content is controlled by the following step UDFs/master step fields that display in the Step Details area of the Record Details screen (see Table 1 and Table 3).

- Experiment Name
- Read 1 Cycles
- Read 2 Cycles
- Workflow
- Reverse Complement Workflow

See the *Bcl2fastq2 Sample Sheet Generation* article for detailed information on the following:

- Sample sheet generation script parameters and usage
- Sample sheet data and configuration options
- Enabling unique FASTQ file names per sequencing run

NovaSeq Run

The following steps outline the sequence of events that occurs when a flow cell is loaded onto the NovaSeq instrument.

User Authentication and Login

User authentication and login to the NovaSeq instrument is achieved via the getSequencerLoginURL endpoint (found at /Illumina/Sequencer/v2/sequencing-run/login). This endpoint returns the URL that the instrument uses to get an access token.

1. The Sequencer API service provides the NovaSeq Control Software (NVCS) with a URL for the Clarity LIMS login screen and the NVCS displays this screen. When you log in, this action automatically authorizes the NVCS to use the account to access Clarity LIMS.
2. Enter credentials.
   - Username and password are passed to the Clarity LIMS server, to ensure that the access is valid.
   - By default, the Sequencer API service tries to contact Clarity LIMS at http://localhost:9080/clarity/. This URL is set by the installation script, but it can be configured with the `clarity.url` property in the `application.yml` file. See *application.yml Properties* on page 773.
3. User is redirected to the instrument login page at /Illumina/Sequencer/v2/sequencer_login.

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- If authentication is successful, the screen displays a success message. The Clarity LIMS server provides the OAuth token and username by returning them in redirect to /Illumina/Sequencer/v2/sequencer_login/Success. This redirect does not reload the page, but it allows the NovaSeq to continue with run setup.
- If authentication fails, you are left on the login screen and a warning message informs them of the failure. A background redirect to /Illumina/Sequencer/v2/sequencer_login/Error informs the NovaSeq of the failure.

Respond to Validation/Recipe Request Call from Instrument

1. The sequencing-run/recipe/novaseq endpoint consumes a POST request with a LibraryContainerI, FlowCellId, and reagents information.
   - LibraryContainerId, FlowCellId, and reagents information are provided by the NovaSeq. LibraryContainerId is required.
   - LibraryContainerId and FlowCellId should match either the library tube ID or flow cell ID of the corresponding containers in Clarity LIMS.
   - If there are no containers or multiple containers, an error is generated.
2. All populated containers whose name matches either the LibraryContainerId or FlowCellId are validated as follows:
   - All samples in the container must be queued for a sequencing run step.
   - All samples must be queued for only one sequencing run step.
   - All samples must be queued for the same sequencing run step.

> You can configure the steps that are considered as 'sequencing run steps' in the novaseq.sequenceStepNames property in the application.yml file. The property is a list and each list entry must be the exact name of a sequencing step, not the name of the underlying master step/process type. See *application.yml Properties* on page 773.

3. After a single container has passed validation, the run setup information (run recipe request) is returned to the NVCS via the API and the run recipe is generated.
   - The run recipe information is read from fields configured on the parent process/master step of the samples in the container. The workflow must include recipe definition in that step.
   - By default, the parent process/master step is the Dilute and Denature (NovaSeq 6000 v3.0.1) or Load to Flowcell (NovaSeq 6000 v3.0.1) step, depending on workflow.
   - The default field values match the names of the fields in the NovaSeq v3.2.1 configuration included in PCWP. The field names are configurable through the recipe.udfNames.* properties. Each of these properties corresponds to an entry in the recipe and the value of a property defines the name of the field that is used to populate that entry in the recipe.
   - It is assumed that this parent process/master step removes any illegal characters from the Experiment Name field.

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- The Clarity LIMS download link to the sample sheet created on the run setup step is used for the SampleSheet value in the recipe. The name of the output of the sample sheet on the setup step can be configured through the recipe.sampleSheet.outputName property (Sample Sheet by default) in the application.yml. See*application.yml Properties* on page 773. If no matching output is found, or no file was attached to the output, then the value of the recipe.sampleSheet.notAvailableValue property (Not Available by default) is used in the recipe.

4. After the recipe is generated, it is returned as a JSON file in the response, in the form defined by the Swagger definition for the API.

Run Recipe Contents

The contents of the run recipe are controlled by the sample sheet contents and the step UDFs/master step fields, configured on the Dilute and Denature (NovaSeq 6000 v3.0.1) and Load to Flowcell (NovaSeq 6000 v3.0.1) steps.

Example of run recipe content is as follows.

```
{
  "run_name": "NVCS16027_SPrime_2x250",
  "run_mode": "S4",
  "workflow_type": "DualIndex",
  "librarytube_id": "NV0025867-LIB",
  "flowcell_id": "",
  "sample_loading_type": "NovaSeqStandard",
  "rehyb": false,
  "paired_end": true,
  "read1": 151,
  "read2": 151,
  "index_read1": 8,
  "index_read2": 8,
  "output_folder": "\\\\network_path\\run_data",
  "samplesheet": "<path to sample sheet on LIMS server>",
  "require_samplesheet_authentication": true,
  "usecustomrecipe": false,
  "customRecipe": null,
  "use_basespace": false,
  "basespace_mode": null,
  "use_custom_read1_primer": false,
  "use_custom_read2_primer": false,
  "use_custom_index_read1_primer": false
}
```

For Dilute and Denature (NovaSeq 6000 v3.0.1) step field configuration details, see Table 7. To see how these fields are populated on the Record Details screen at run time, see Table 9.

For Load to Flowcell (NovaSeq 6000 v3.0.1) step field configuration details, see Table 14. To see how these fields are populated on the Record Details screen at run time, see Table 16.

Respond to Sequencing Started Call from Instrument

Matching container for the run (library tube or flow cell) is found, and contents are validated to be queued for the correct step.

An error is thrown and the step is not started if no matching container is found, or if the samples in the container are incorrectly queued. This validation is also done during the run recipe request immediately prior, so this error case should not be encountered.

1. Reagent kits that are configured for the Sequencer API are checked against the configuration for the step to be run:
   - If a kit is configured for the API but does not exist in Clarity LIMS, it is created.
   - If a kit exists, but is not enabled on the step to be run, the step configuration is updated to enable it.
     - If a kit that is to be enabled is Archived, it is set to Active.
     - If a kit that is to be enabled is in Pending status, it is set to Active.
   - If any kits enabled on the step are not configured for the Sequencer API, a warning is logged and the step is unable to be completed by the API without user intervention.
   - If any kits provided in the recipe request are not configured for the Sequencer API, they are ignored and a warning is logged during lot tracking.

   The number of replicates for the step is determined by the type of flow cell being used in the run.
   - For Standard mode, this number is based on the number of lanes in the flow cell (this is stored as part of the Sequencer API configuration).
   - For XP mode, this number is always one replicate, because there is already one input tracked for each lane.
2. The automated sequencing step is started.
3. Reagent lots are attached to the step.
   - The Flow Cell and Library Tube reagents in the request are ignored. Information from these reagents are tracked in Clarity LIMS as containers and UDFs.
   - Any other reagents in the request are handled as follows:
     - If the reagent is not configured to be tracked, a warning is logged and the reagent is ignored.
     - Existing reagent lots are looked up by kit name (determined by Sequencer API configuration), name (the serial number in the request), and lot number (the lot number in the request).
     - If one or more matching reagent lots exists in Clarity LIMS, the newest one is used:

- If the expiry date does not match the expiry in the request, or if the status is not set to Active, the lot is updated to have the correct expiry date and an Active status.

- Otherwise a new lot is created with:
  - Reagent Kit—Matching kit as determined from Sequencer API configuration and previous step
  - Name—Serial number in request
  - Lot Number—Lot number in request
  - Expiry—Expiry date in request
  - Status—Active

  If any reagents were expected to be in the request but were not, these reagents are logged. This action prevents the step from being completed without user intervention.

4. Step UDFs/master step fields are updated with the run information parsed from the request. See *Respond to Record InterOps Metrics Call from Instrument* on page 771.
5. Any important events during this request are logged into the Sequencer API log file and in the Sequencing Log multiline UDF/field on the step (assuming the step was started successfully). See *Respond to Record InterOps Metrics Call from Instrument* on page 771.

Respond to Record InterOps Metrics Call from Instrument

When the metrics endpoint receives a POST, it does as follows.

Looks for a matching library tube or flow cell in Clarity LIMS.

Finds the in progress sequencing step for that container.

When the step is found, the run metrics in the request are written to container UDFs/global fields in Clarity LIMS.

Respond to Sequencing Completed Call from Instrument

If the sequencing run was successful, after the metrics have been recorded, the API call for Sequencing Completed is received. Information is matched by Sequencing Container and the following steps occur in Clarity LIMS:

1. The Current Cycle, Current Read, and Status UDFs/Master Step fields are updated.
2. The multiline Sequencing Log UDF/master step field is updated.
3. A success message is recorded in the log file, at the info level:

```
Sequencing run with Run ID {runId} of Step '{step.name}' ({step.limsid})
completed successfully.
```

4. Clarity LIMS waits for any automations that are triggered automatically as it advances the step.
5. By default, Clarity LIMS assigns the next steps on the outputs to move them on to the first configured next step. Or, if there are no next steps, to complete the protocol.

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Respond to Sequencing Errored Call from Instrument

If the sequencing run was unsuccessful, the API call for Sequencing Error is received. Information is matched by Sequencing Container and the following occurs in Clarity LIMS:

1. The Current Cycle, Current Read, and Status UDFs/Master Step fields are updated.
2. The multiline Sequencing Log UDF/master step field is updated.
3. A failure message is recorded in the log file, at the error level:

```
Step '${Step.name}' (${Step.limsid}) failed with Run ID ${runId}.
```

4. The step is left at the Record Details screen.

Respond to Sequencing RunEndedByUser Call from Instrument

If the sequencing run is ended, the API call for Sequencing RunEndedByUser is received. Information is matched by Sequencing Container and the following occurs in Clarity LIMS:

1. The Current Cycle, Current Read, and Status UDFs/Master Step fields are updated.
2. The multiline Sequencing Log UDF/Master Step field is updated.
3. The following message is recorded in the log file, at the warning level:

```
"Sequencing run with Run ID ${status.runInfo.runId} of Step
'${step.configuration.name}' (${step.limsid}) aborted by user
${status.runInfo.userName}."
```

4. The step is left at the Record Details screen.

Components Installed with this Integration

The following sections describe the various components that are installed by default as part of this integration. These components include files, properties, reagent categories/label groups, reagent kits, and containers. Information on installed workflows, protocols, steps, and automation points is provided in the Workflows, Protocols, and Steps section.

Illumina NovaSeq Integration v3.2.2 is distributed as an RPM package: BaseSpaceLIMS-sequencer-api. This RPM package must be installed on the Clarity LIMS server.

The BaseSpaceLIMS-sequencer-api RPM installs the following items:

- Sequencer API WAR file
- Configuration file: `application.yml`
- Two configuration scripts: `configure_sequencer_api_proxy.sh` and `configure_sequencer_api_application.sh`

The NovaSeq v3 configuration is delivered through the Preconfigured Workflows Package (PCWP) v1.2.2, which requires installation of the NGS Extensions Package v5.18 or later.

- If the NGS Extensions Package is not already installed, or if a version earlier than v5.18 is installed, the latest version is installed by default with the NovaSeq integration. See Release Notes for details.
- If NGS Extensions Package v5.18 or later is already installed, the upgrade is not forced or required.

The RPM package installs the components listed in Table 18.

Table 213 Table 18: Illumina NovaSeq 6000 Integration RPM Components

Files Installed	Location and Description
Illumina#Sequencer#v2.war	/opt/gls/clarity/tomcat/current/webapps War file for NovaSeq v3.2.2 API
configure_sequencer_api_application.sh	/opt/gls/clarity/config/ Script that configures the Sequencer API application through its external application.yml file
configure_sequencer_api_proxy.sh	/opt/gls/clarity/config/ Script that configures the proxy to allow communication with the Sequencer API (NovaSeq v3.2.2 API) application
sequencer-api.conf	/etc/httpd/clarity/ proxy setting to communicate with Clarity LIMS

application.yml Properties

The `application.yml` file is located at opt/gls/clarity/extensions/sequencing-api/.

- All properties are configured automatically during installation, but you can also configure them by editing the `application.yml` file and then restarting Tomcat.
- All custom field names, both for recipe and run information, are represented by properties in the file.
- For all custom fields, the names (values in this file) can be changed. However, the property names cannot be changed, removed, or added to. For example, you can change Loading Workflow Type but you cannot change recipe.udfNames.sampleLoadingType.

Table 19 lists the properties it contains.

Table 214 Table 19: application.yml Properties Installed

Property	Description
spring.profiles.active	Tells the application that it is running deployed in Tomcat. Default value: tomcat (Do not change the default value)
clarity.url	Base URL that the Sequencer API service uses to contact Clarity LIMS. The installation script (configure_ sequencer_api_application) prompts for this property.

Property	Description
clarity.username	Username to be used when communicating with Clarity LIMS.
clarity.password	Encrypted password to be used when communicating with Clarity LIMS.
security.signing-key	Private key that should be used when signing/validating OAuth tokens. Changing this property invalidates any issued tokens.
security.token-expiry	Specifies (in hours) the expiry period for login tokens issued by the Sequencer API. Default value: 88
novaseq.sequenceStepNames	List of NovaSeq sequencing run step names for which the integration may find samples queued. • Names must be an exact match to the name of the step in Clarity LIMS(not the master step/process type). • Each line should be indented to the same point and start with a dash (-). Default value: • AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0.1)
novaseq.flowcells	List of supported NovaSeq flow cell types. • Type names exactly match the values used by the NVCS run status requests. • To add more types, provide both type and lanes properties indented to the same point and starting with a dash (-). Default value: `- type: "SP"lanes: 2- type: "S1"lanes: 2- type: "S2"lanes: 2- type: "S4"lanes: 4`

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Property	Description
novaseq.reagents	List of supported reagent kits to be tracked on sequencing steps. • lotName—The name of the lot as it appears in the run started request. • kitName—The name of the corresponding reagent kit in Clarity LIMS that the lot should be tracked as. The name may differ in the NVCS request vs. the Clarity LIMS configuration. Default value: `- lotName: "Buffer"kitName: "Buffer Cartridge"- lotName: "Cluster"kitName: "Cluster Cartridge"- lotName: "SBS"kitName: "SBS Cartridge"`
recipe.udfNames	Configures the names of the UDFs/fields that are used in the run recipe (see *Run Recipe Contents* on page 769 for more information). • Each value must be enclosed in quotes. Example: recipe.udfNames.runMode "Run Mode" • Value provided for sampleLoadingType must match the value provided in the run configuration. Example: recipe.udfNames.sampleLoadingType "Loading Workflow Type"
recipe.sampleSheet.outputName recipe.sampleSheet.notAvailableValue	Configures the name of the sample sheet file placeholder on the step where run recipe information is populated, and the property value to use when the file is not found.
run.stepUdfNames	Configures the step UDF/master step field names that are used when recording run results (see *Step UDFs/Custom Fields* on page 764 for more information). • Each value must be enclosed in quotes. Example: run.stepUdfNames.runID "Run ID" • Value provided for sampleLoadingType must match the value provided in the run configuration. • Example: run.stepUdfNames.sampleLoadingType "Loading Workflow Type". It is expected that the run step field and the field on the input derived sample share a name.

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Property	Description
run.metricUdfNames	Configures the result file UDFs/measurement global fields names that are used when recording run results. These fields capture the run data (see *Global UDFs/Custom Fields* on page 765 for more information).
run.autoComplete	Determines whether the sequencing run step should be completed automatically after parsing the run metrics on a run (see also run.autoCompleteOnlyAtSuccess). Default value: true See *Configuring autoComplete Properties* on page 777.
run.autoCompleteOnlyAtSuccess	Determines whether the sequencing run step should be: • Autocompleted when the run status is RunCompletedSuccessfully (ifautoCompleteOnlyAtSuccess is true) - or - • Autocompleted regardless of the run status (if autoCompleteOnlyAtSuccess is false). Default value: true See *Configuring autoComplete Properties* on page 777.

Reagent Categories/Label Groups

- TruSeq HT Adapters v2 (D7-D5)

Reagent Kits

- Buffer Cartridge
- Cluster Cartridge
- DPX1
- DPX2
- DPX3
- NaOH
- Resuspension Buffer (RSB)
- SBS Cartridge
- Tris HCl

Container Types

- Library Tube
- SP
- S1

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- S2
- S4

This integration supports:

- Library tube with barcode provided in the following format:
  - [A-Za-z]{2}[0-9]{7}-[A-Za-z]{3}
  - Example: AB1234567-XYZ
- SP, S1, S2, or S4 flow cell, with barcode provided in one of the following formats:
  - SP and S1 flow cell: [A-Za-z0-9]{5}DRX[XY2357]
  - S2 flowcell: [A-Za-z0-9]{5}DMX[XY2357]
  - S4 flow cell: [A-Za-z0-9]{5}DSX[XY2357]
  - Example: H1991DMXX

NVCS Configuration

For details on configuring NVCS for integration with Clarity LIMS, contact the Clarity LIMS Support team.

Routing Script Requirements

The requirements for the routing script functionality are as follows.

- On the steps that use the routing script Define Run Format (NovaSeq 6000 v3.0.1) and Dilute and Denature (NovaSeq 6000 v3.0.1), the Next Step for all samples must be set to Remove from workflow. This value is set by a script. Do not change this value in the Assign Next Steps screen.
- In Clarity LIMS v5, the following settings are required and must not be changed on the protocol configuration screen:
  - In the Next Steps section for the last step in the protocol, the method of assigning the next step must be set to Automatic.
- In Clarity LIMS v4, the following setting in the protocol configuration area is required and must not be changed for any protocol steps on which the Routing Script automation is configured:
  - On the Next Steps configuration tab, the Next Steps are set by a script toggle switch must be set to Yes.

Configuring autoComplete Properties

As of NovaSeq Integration v3.2.2, the `application.yml` configuration file contains two additional properties:

- run.autoComplete
- run.autoCompleteOnlyAtSuccess

These properties determine the conditions under which the last step of the NovaSeq 6000 workflow—AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0.1)—is automatically completed:

- Only when the run status is RunCompletedSuccessfully (default behavior)

  -or -

- Regardless of the run status

By default, both properties are set to true and the step only completes if the run is successful. If the run fails or is aborted, manually complete the step in Clarity LIMS.

To autocomplete the step regardless of the result of the sequencing run, change the autoCompleteOnlyAtSuccess property value to false.

Enabling/Disabling autoComplete Properties

1. Open the `application.yml` file located at /opt/gls/clarity/extensions/sequencer-api/application.yml:

```
vi /opt/gls/clarity/extensions/sequencer-api/application.yml
```

2. Edit the value of the autoCompleteOnlyAtSuccess property as required.
3. Save the file.

Table 20 shows how the combined value of the run.autoComplete and run.autoCompleteOnlyAtSuccess properties affects the autocomplete behavior of the sequencing step.

Table 215 Table 20: run.autoComplete and run.autoCompleteOnlyAtSuccess Value Matrix

run.autoComplete Property Value	run.autoCompleteOnlyAtSuccess Property Value	Outcome
True (default)	True (default)	• Step autocompletes only if sequencing run is successful. • If run is not successful, step does not autocomplete and run details are recorded in Clarity LIMS.
True	False	• Step autocompletes, regardless of the run status.
False	True	• Step does not autocomplete. • Run details are recorded in Clarity LIMS.
False	False	

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Rules and Constraints

- The workflow configuration contains several validation checks. To make that the calculations work properly, it is important not to disable any of this validation logic. The validation checks determine:
  - Which samples, and how many, can enter each step together.
  - Which samples, and how many, can be pooled together.
- The library tube ID must be unique. There should not be multiple library tube containers in the system with the same name.
- Reagent labels (indexes) must be unique.
- Only controls are permitted as unindexed samples. All other unindexed samples and pools are not permitted.
- For sample sheet generation constraints, refer to the *Bcl2fastq2 Sample Sheet Generation* article.
- Do not manually start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0.1) step. This step is fully automated and the sequencing service does not update samples correctly if they have been manually started.
- For the automated run to start successfully, the Validate Run Setup and Generate Sample Sheet button must be selected.

Resources

- *NovaSeq 6000 Sequencing System Guide*

NovaSeq 6000 Integration v3.2.2 Validation and Troubleshooting

This guide explains how to validate the installation of the Illumina NovaSeq 6000 Integration Package v3.2.2.

The validation process involves:

- Running samples through the Library Prep Validation v1.0.1 workflow.
  - The workflow contains a single-step protocol that models the library prep required to produce normalized libraries. At the end of the step, the normalized libraries are advanced to the workflow selected.
- Running normalized libraries through the NovaSeq 6000 v3.0.1 workflow. This action validates the following:
  - Successful routing of samples from the Run Format (NovaSeq 6000 v3.0.1) step to the NovaSeq Standard (NovaSeq 6000 v3.0.1) or NovaSeq Xp (NovaSeq 6000 v3.0.1) step.
  - Automated generation of a sample sheet for use with bcl2fastq2 v2.20 analysis software. This file is automatically uploaded to the sequencing instrument via the Sequencer API.

- Automatic validation of run setup information. This information is uploaded to the NovaSeq Control Software (NVCS) via the Sequencer API and is used to create the run recipe and initiate the run.
- Automated tracking of the NovaSeq sequencing run and parsing of run statistics into Clarity LIMS, via the Sequencer API.

Before running the validation steps below, these steps assume that you have installed the Illumina NovaSeq 6000 Integration Package v3.2.2 and have imported the default Clarity LIMS configuration.

Validation Steps

Activate Workflow, Create Project, Add and Assign Samples

The following steps set up Clarity LIMS in preparation for running samples through the Library Prep Validation v1.0.1 and NovaSeq 6000 v3.0.1 workflows.

Step 1: Activate the Workflow

In the Clarity LIMS configuration area, activate the Library Prep Validation v1.0 and NovaSeq 6000 v3.0 workflows.

Step 2: Create a Project, Add Samples

1. On the Projects and Samples screen, create a project and add samples to it.
2. Assign your samples to the Library Prep Validation v1.0 workflow.

Library Prep Protocol: Library Prep Validation v1.0.1

This single-step protocol models the library prep required to produce normalized libraries that are ready for the NovaSeq 6000 v3.0.1 workflow.

Step 1: Run Library Prep Validation v1.0.1

1. In Lab View, locate the Library Prep Validation v1.0.1 protocol. The Library Prep Validation v1.0.1 step opens.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Placement screen:
   a. In the Placed Samples area on the right in the container field, scan or enter the barcode of the destination container.
   b. Select the samples from the Samples to be Placed area on the left side, then drag them to the container wells on the right side.

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c. Select Add Labels.

5. On the Add Labels screen, the Labels to Add list is populated with labels from the TruSeq HT Adapters v2 (D7-D5) label group. This label group is the only one configured in the out-of-the-box integration. It is used by default in this step.
   a. Select labels on the left side, and drag them to the container on the right side to assign them to placed samples.
   b. Select Record Details.
6. On the Record Details screen:
   a. In the Sample Details table, enter values for Normalized Molarity. Type values individually or use the Copy/Paste functionality to enter multiple values from a spreadsheet.
   b. The Sequencing Instrument column indicates the instrument to be used for the sequencing run. Select NovaSeq 3.0.
   c. Populate the fields in this column using one of the following methods:
      - To populate fields individually for each sample, select from the drop-down list in each row.
      - To populate fields all at one time, use the Copy/Paste functionality to enter multiple values from a spreadsheet.

   Alternatively, select inside the table and press Ctrl + A to select all rows. At the top of the table, select Sequencing Instrument and then select NovaSeq 3.0 from the adjacent drop-down list. Select Apply.

   If you are working with many samples, you can use Copy/Paste functionality to enter multiple values from a spreadsheet.

   a. Select Copy Samples to copy the sample information from Clarity LIMS.
   b. Open a blank Excel document and paste the sample information into the spreadsheet.
   c. In the spreadsheet, enter the Normalized Molarity and Sequencing Instrument values. Select the entire spreadsheet, including the headers (Ctrl + A), and copy (Ctrl + C) the information.
   d. In Clarity LIMS, select Paste Samples. Paste the information (Ctrl + V) into the Update Table window, then select Update to paste into the Sample Details table.
7. Select Next Steps.
   This selection triggers the Next Steps automation, which sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step successfully.
8. On the Assign Next Steps screen in the Samples table, the Next Step for all samples is prepopulated with Remove from workflow.

   Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.

9. Select Finish Step.

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On exit from the step, the Routing Script automation is triggered. This automation assigns samples to the first step of the NovaSeq 6000 v3.0.1 workflow - Define Run Format (NovaSeq 6000 v3.0.1). This step is the only step in Protocol 1: Run Format (NovaSeq 6000 v3.0.1).

Protocol 1: Run Format (NovaSeq 6000 v3.0.1)

This protocol includes a single step: Define Run Format (NovaSeq 6000 v3.0.1). The step allows for the assignment of per sample values for Loading Workflow Type, Normalized Molarity, Flowcell Type, and Final Loading Concentration (pM). At the end of the step, samples are routed to the NovaSeq Standard or NovaSeq Xp protocol, according to the selected Loading Workflow Type.

Step 1: Run Define Run Format (NovaSeq 6000 v3.0.1)

1. In Lab View, locate the Run Format (NovaSeq 6000 v3.0.1) protocol. The samples are queued for the Define Run Format (NovaSeq 6000 v3.0.1) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Record Details screen in the Sample Details table, populate the following fields (values can vary across samples):
   - Loading Workflow Type—Select NovaSeq Standard or NovaSeq Xp from the drop-down list.
   - Flowcell Type—Select SP, S1, S2, or S4.
   - Final Loading Concentration (pM)—Select from the two preset options - 225 (for PCR-free workflows) or 400 (for Nano workflows), or you may enter a different value.
   - Normalized Molarity (nM)—These values are copied over from the previous step. If this column is not populated during library prep, enter the values here.
5. Select Next Steps. This selection triggers the Next Steps automation, which does the following actions:
   - Sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step successfully.
   - Calculates the Minimum Molarity.
   - Checks Normalized Molarity value. For samples with no Normalized Molarity value (ie, an empty value, not including 0), it generates an error message stating that the field cannot be empty.
   - Compares each the Normalized Molarity value of each sample with the Minimum Molarity value.
6. On the Assign Next Steps screen:
   - In the Sample Details table, the Next Step for all samples is prepopulated with Remove from workflow, regardless of the Loading Workflow Type.

   Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.

- For samples whose Normalized Molarity value was found to be lower than the Minimum Molarity value, the Loading Workflow Type is set to Remove from workflow. A message is also recorded in the Warning field for the sample.
  At this point you have two options:
  - Return to the Record Details screen and adjust the Normalized Molarity value so that it equals or exceeds the Minimum Molarity value. Set the Loading Workflow Type to NovaSeq Standard or NovaSeq Xp, as applicable.
  - Complete the protocol without correcting the Normalized Molarity value. In this case, the samples in question are removed from the Clarity LIMS workflow.

7. Select Finish Step. The Routing Script automation is triggered:
   - Samples whose Loading Workflow Type is set to Remove from workflow (ie, Normalized Molarity value is lower than the Minimum Molarity) are removed from the Clarity LIMS workflow.
   - Samples whose Loading Workflow Type is NovaSeq Standard are routed to the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.0.1) step. This step is the first of two steps in *Protocol 2: NovaSeq Standard (NovaSeq 6000 v3.0.1)* on page 783.
   - Samples whose Loading Workflow Type is set to NovaSeq Xp are routed to the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.0) step. This step is the first of three steps in *Protocol 3: NovaSeq Xp (NovaSeq 6000 v3.0.1)* on page 788.

Protocol 2: NovaSeq Standard (NovaSeq 6000 v3.0.1)

In this protocol, samples are pooled and added to the library tube in preparation for the NovaSeq run. The protocol contains two steps:

1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.0.1)
2. Dilute and Denature (NovaSeq 6000 v3.0.1)

Step 1: Run Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.0.1)

1. In Lab View, locate the NovaSeq Standard (NovaSeq 6000 v3.0.1) protocol. The samples are queued for the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.0.1) step.
2. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Pooling screen:
   a. Create a pool by dragging samples into the Pool Creator.

      Only create one pool.

   b. Type a name for the pool or accept the default name (Pool #1).

c. Select Record Details.

On exit from the Pooling screen, the Validate Inputs Flowcell Type and Single Pool is triggered. The automation checks the following details:

- All samples in the pool have been assigned the same Flowcell Type.
- Only one pool has been created.

5. On the Record Details screen, the Step Details area contains two required fields:
   - Number of Flowcells to Sequence—This value is used in volume calculations to make sure that volumes are sufficient for the number of times the pool is sequenced.
   - Minimum Per Sample Volume (µl)—This value is used to calculate how much of each sample is included in the pool. The field is prepopulated with the configured default value (5 µl). The value may be edited if needed.

   Assuming the default Minimum Per Sample Volume (µl) value of 5, for a given batch:
   a. If the smallest Per Sample Volume (µl) value is less than 5, Clarity LIMS automatically assigns a value of 5 to the Adjusted Per Sample Volume (µl) field.
   b. Clarity LIMS then adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.
6. In the Sample Details table, select the pool icon to view details on the pool composition.
7. Select Calculate Volumes. This selection triggers the Calculate Volumes automation, which performs volume calculations based on the selected Flowcell Type:
   - Calculates Bulk Pool Volume (µl) value.
   - Sets the value of the Bulk Pool Volume (µl) field, based on the selected Flowcell Type.
   - Calculates the Per Sample Volume (µl) value to be added to the pool.
   - Calculates the Total Sample Volume (µl) value.
   - If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) value.
   - Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
   - Populates the Volume of Pool to Denature (µl), NaOH Volume (µl) and Tris-HCl Volume (µl) columns of the Sample Details table. Values are set by a script and are not editable.
   - The automation also generates the Calculation File (*csv) and attaches it to the step. This file contains volume information about the pool and the individual samples it contains. Select the file to download it and then open it in Excel.
8. Select Next Steps. This selection triggers the Set Next Step automation. This automation sets the next step for samples to ADVANCE and advances them to the next step in the protocol: Dilute and Denature (NovaSeq 6000 v3.0.1).

   On the Assign Next Steps screen, the next step for samples is already set to the next step in the workflow: Dilute and Denature (NovaSeq 6000 v3.0.1).

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9. Select Finish Step.

At the end of this step, the pool of samples automatically advances to the Dilute and Denature (NovaSeq 6000 v3.0.1) step.

Step 2: Run Dilute and Denature (NovaSeq 6000 v3.0.1)

1. In Lab View, locate the NovaSeq Standard (NovaSeq 6000 v3.0.1) protocol. The samples are queued for the Dilute and Denature (NovaSeq 6000 v3.0.1) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work. The Validate Single Input automation is triggered. This automation checks that there is only one container input to the step.
4. On the Placement screen:
   a. Drag the pool into the library tube in the Placed Samples area.
   b. Scan or type the barcode of the library tube into the Library Tube field.
   c. Select Record Details.
   On exit of the Placement screen, the Validate Library Tube Barcode automation checks that the library tube barcode conforms to the barcode mask [A-Za-z]{2}[0-9]{7}-[A-Za-z]{3}. If not, an error message is displayed. This automation also copies the Flowcell Type and Loading Workflow Type values from step inputs to outputs.
5. On the Record Details screen, the Reagent Lot Tracking section tracks the NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. Add and activate lots for these reagents.
   To add and activate reagent lots:
   a. In a new browser tab, open an additional instance of Clarity LIMS.
      - Clarity LIMS v5: Navigate to the Consumables configuration screen.
      - Clarity LIMS v4: Navigate to the Reagents and Controls screen.
   b. Select the NaOH reagent kit and select New Lot.
   c. Enter the lot details and select Save.
   d. Set the Status of Reagent Lot toggle switch to Active.
   e. Repeat this process to add and activate Resuspension Buffer and Tris-HCl reagent lots.
   f. Return to the original working browser tab and refresh the page.
6. In the Reagent Lot Tracking section, select from the active lots displayed in each drop-down list.
7. The fields displayed on the Record Details screen are used to set up the run and generate the sample sheet. Some of these fields are autopopulated and some must be manually completed.

Table 216 Fields on the Record Details Screen of Denature & Dilute (NovaSeq 6000 v3.0.1) Step

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. No spaces.
BaseSpace Sequence Hub Configuration	Select from preset options: Not Used, Run Monitoring Only, or Run Monitoring and Storage.
Workflow	Automatically populated with preset - GenerateFASTQ.
Workflow Type	Select from preset options: No Index, Single Index, Dual Index, or Custom.
Index Read 1	Select from preset options: 0, 6, or 8 - or type a value between 0 and 20.
Index Read 2	Select from preset options: 0, 6, or 8 - or type a value between 0 and 20.
Paired End	Select from preset options: True or False.
Read 1 Cycles	Select from preset options: 251, 151, 101, or 51 - or type a value between 1 and 251. *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Read 2 Cycles	Select from preset options: 251, 151, 101, or 51 - or type a value between 1 and 251. *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Use Custom Read 1 Primer	Select if applicable.
Use Custom Read 2 Primer	Select if applicable.
Use Custom Index Read 1 Primer	Select if applicable.
Reverse Complement Workflow	Select Yes (by default) for v1.5 reagents in NovaSeq Control Software v1.7 and onwards. Select No for v1 reagents.
Use Custom Recipe	Select if applicable.
Custom Recipe Path	If the Use Custom Recipe option is selected, enter the path to the custom recipe file to be used.
UMI - Read 1 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 1. Leave blank if not applicable.

Field	Value
UMI - Read 2 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 2. Leave blank if not applicable.
UMI - Read 1 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.
UMI - Read 2 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.
Paired End	Select if applicable.
Output Folder	Enter network path for sequencing run folder. For example: \\networkshare\run_data

8. On the Record Details screen, select Validate Run Setup and Generate Sample Sheet. This selection triggers the automation script, which does the following actions:
   - Validates the parameters entered on the Record Details screen.
   - Generates the sample sheet and attaches it to the placeholder in the Files area of the Record Details screen.
9. Select Next Steps.
   On the Assign Next Steps screen, the Next Step field is prepopulated with Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step successfully.

   Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.

10. Select Finish Step.
    On exit from the step:
    - The Routing Script automation is triggered and samples are routed to Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0.1).
    - In Lab View, the pool of samples is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0.1) step.

At this point in the workflow, the user interaction ends. Proceed to *Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0.1)* on page 793

Do not add samples to the Ice Bucket or start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0.1) step. The integration does this step automatically.

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Protocol 3: NovaSeq Xp (NovaSeq 6000 v3.0.1)

In this protocol, samples are pooled and added to lanes on the NovaSeq flow cell. The flow cell type is determined by the option selected in the Define Run Format (NovaSeq 6000 v3.0.1) step.

The protocol contains three steps:

1. Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.0.1)
2. Dilute, Denature, and ExAmp (NovaSeq 6000 v3.0.1)
3. Load to Flowcell (NovaSeq 6000 v3.0.1)

Step 1: Run Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.0.1)

1. In Lab View, locate the NovaSeq Standard (NovaSeq 6000 v3.0.1) protocol. The samples are queued for the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.0.1) step.
2. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Pooling screen:
   a. Create a pool by dragging samples into the Pool Creator.

      Only create one pool.

   b. Type a name for the pool or accept the default name (Pool #1).
   c. Select Record Details.
5. On exit from the Pooling screen, the Validate Inputs Flowcell Type and Single Pool is triggered. The automation checks the following details:
   - All samples in the pool have been assigned the same Flowcell Type.
   - Only one pool has been created.
6. On the Record Details screen, the Step Details area contains two required fields:
   - Number of Lanes to Sequence—The value entered in this field is used in volume calculations to make sure that volumes are sufficient for the number of times the pool is sequenced.
   - Minimum Per Sample Volume (µl)—The value in this field is used to calculate how much of each sample is included in the pool. The field is prepopulated with the configured default value (5 µl). The value may be edited if necessary.

   Assuming the default Minimum Per Sample Volume (µl) value of 5 for a given batch:
   a. If the smallest Per Sample Volume (µl) value is less than 5, Clarity LIMS automatically assigns a value of 5 to the Adjusted Per Sample Volume (µl) field.
   b. Clarity LIMS then adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.
7. In the Sample Details table, select the pool icon to view details on the pool composition.

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8. Select Calculate Volumes. This selection triggers the Calculate Volumes automation, which performs volume calculations based on the selected Flowcell Type:
   - Calculates Bulk Pool Volume (µl) value.
   - Calculates the Per Sample Volume (µl) value to be added to the pool.
   - Calculates the Total Sample Volume (µl) value.
   - If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) value.
   - The automation also populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
   - The automation also generates the Calculation File (*csv) and attaches it to the step. This file contains volume information about the samples and RSB buffer to add to the pool. Select the file to download it and then open it in Excel.
9. Select Next Steps. This selection triggers the Set Next Step automation, which:
   - Copies the Flowcell Type values from the step inputs to the step outputs.
   - Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol - Dilute, Denature, and ExAmp (NovaSeq 6000 v3.0.1).

   On the Assign Next Steps screen, the next step for samples is already set to the next step in the workflow: Dilute, Denature, and ExAmp (NovaSeq 6000 v3.0.1).
10. Select Finish Step.

At the end of this step, the pool of samples automatically advances to the Dilute, Denature, and ExAmp (NovaSeq 6000 v3.0.1) step.

Step 2: Run Dilute, Denature and ExAmp (NovaSeq 6000 v3.0.1)

1. In Lab View, locate the NovaSeq Xp (NovaSeq 6000 v3.0.1) protocol. The pool of samples is queued for the Dilute, Denature, and ExAmp (NovaSeq 6000 v3.0.1) step.
2. Add the pool to the Ice Bucket and select View Ice Bucket.
3. [Optional] On the Ice Bucket screen, set the number of derivatives to create (they are placed into the flow cell lanes) and select Begin Work.
4. On entry to the Record Details screen, the Calculate Volumes automation is triggered.
   This automation sets the following values based on the selected Flowcell Type:
   - Bp Aliquot Volume (µl)
   - NaOH Volume (µl)
   - Tris-HCl Volume (µl)
   - DPX1 Volume (µl)
   - DPX2 Volume (µl)
   - DPX3 Volume (µl)

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- Mastermix per Lane (µl)
- The automation also populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
- The automation also generates the Calculation File (*csv) and attaches it to the step. This file contains information about the DPX Mastermix volume, and the volume of Mastermix, NaOH, and Tris-HCl to add per working pool.

5. On the Record Details screen, the Reagent Lot Tracking section tracks the DPX1, DPX2, DPX3, NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. Add and activate lots for these reagents.
   To add and activate reagent lots:
   a. In a new browser tab, open an additional instance of Clarity LIMS.
      - Clarity LIMS v5: Navigate to the Consumables configuration screen.
      - Clarity LIMS v4: Navigate to the Reagents and Controls screen.
   b. Select one of the reagent kits listed previously (NaOH shown later) and select New Lot.
   c. Enter the lot details and select Save.
   d. Set the Status of Reagent Lot toggle switch to Active.
   e. Repeat this process to add and activate the remaining reagent lots.
   f. Return to your original working browser tab and refresh the page.
6. On the Record Details screen:
   - In the Reagent Lot Tracking section, select from the active lots displayed in each drop-down list.
   - In the Step Details area, the DPX1, DPX2, and DPX3 reagent volume values are already populated. These values are set by a script and are not editable while running the step.
7. In the Sample Details table, select the pool icon to view details on the working pool composition.
   - The BP Aliquot, Mastermix per lane, NaOH, and Tris-HCl volume values for each working pool are already populated. These values are set by a script and are not editable while running the step.
   - The Flowcell Type and Loading Workflow Type columns are already populated.
   - The working pool number is appended to the bulk pool name. This feature allows you to identify quickly which working pools are derived from the same bulk pool.
   - In the Files area, select the Calculation File (*csv) to open it and view details on the DPX Mastermix volume, and the volume of Mastermix, NaOH. and Tris-HCl to add per working pool.
8. Select Next Steps.
   On the Assign Next Steps screen, the next step is already set to Load to Flowcell (NovaSeq 6000 v3.0.1).
9. Select Finish Step.

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Step 3: Run Load to Flowcell (NovaSeq 6000 v3.0.1)

1. On the Ice Bucket screen:
   a. In the Container Options panel, select the appropriate flow cell type from the Destination Container drop-down list.
   b. Select Begin Work.
2. The Validate Inputs and Selected Container automation checks the following details:
   - The Flowcell Type field has been set to a valid value (SP, S1, S2, or S4) and all inputs have the same value for this field.
   - The container type selected matches the value in the Flowcell Type field.
   - The number of outputs matches the number of lanes on the selected flow cell type. If validation fails, an error message states that the number of working pools does not match the number of lanes available on the flow cell.
3. On the Placement screen:
   a. Drag the pools from the left of the screen over into the Placed Samples area on the right.
   b. Scan or type the barcode of the flow cell into the Flow Cell field.
   c. Select Record Details.

   On exit of the Placement screen, the Validate Flowcell Barcode automation checks that the container barcode conforms to the barcode mask for the selected Flowcell Type. See the following table. This automation also copies the Flowcell Type and Loading Workflow Type field values from step inputs to outputs.

Flow Cell Type	Barcode Mask
SP	[A-Za-z0-9]{5}DRX[XY2357]
S1	[A-Za-z0-9]{5}DRX[XY2357]
S2	[A-Za-z0-9]{5}DMX[XY2357]
S4	[A-Za-z0-9]{5}DSX[XY2357]

4. The fields displayed on the Record Details screen are used to set up the run and generate the sample sheet.
   Some of these fields are autopopulated and some must be completed manually (see the following table for details).

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. No spaces.
BaseSpace Sequence Hub Configuration	Select from preset options: Not Used, Run Monitoring Only, or Run Monitoring and Storage.

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Field	Value
Workflow	Automatically populated with preset - GenerateFASTQ.
Workflow Type	Select from preset options: No Index, Single Index, Dual Index, or Custom.
Index Read 1	Select from preset options: 0, 6, or 8 - or type a value between 0 and 20.
Index Read 2	Select from preset options: 0, 6, or 8 - or type a value between 0 and 20.
Paired End	Select from preset options: True or False.
Read 1 Cycles	Select from preset options: 251, 151, 101, or 51 - or type a value between 1 and 251. *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Read 2 Cycles	Select from preset options: 251, 151, 101, or 51 - or type a value between 1 and 251. *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Use Custom Read 1 Primer	Select if applicable.
Use Custom Read 2 Primer	Select if applicable.
Use Custom Index Read 1 Primer	Select if applicable.
Reverse Compliment Workflow	Select Yes (by default) for v1.5 reagents in NovaSeq Control Software v1.7 and onwards. Select No for v1 reagents.
Use Custom Recipe	Select if applicable.
Custom Recipe Path	If you selected the Use Custom Recipe option, enter the path to the custom recipe file to be used.
UMI - Read 1 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 1. Leave blank if not applicable.
UMI - Read 2 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 2. Leave blank if not applicable.
UMI - Read 1 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.

Field	Value
UMI - Read 2 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.
Output Folder	Enter network path for sequencing run folder. For example: \\networkshare\run_data
Library Tube Barcode	Scan the library tube barcode.

5. Select Validate Run Setup and Generate Sample Sheet. This selection triggers the automation script, which does the following actions:
   - Validates the parameters entered on the Record Details screen.
   - Generates the sample sheet and attaches it to the placeholder in the Files area of the Record Details screen.
6. Select Next Steps.
   On the Assign Next Steps screen, the Next Step field for samples is prepopulated with Mark protocol as complete.
7. Select Finish Step.

At this point in the workflow, the user interaction ends. The flow cell is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0.1) step.

Proceed to *Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0.1)* on page 793.

Do not add samples to the Ice Bucket or start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0.1) step. The integration does this step automatically.

Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0.1)

This protocol contains a single fully automated step: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0.1).

The integration starts the step automatically and data from the run is parsed back into Clarity LIMS. No user interaction is required. However, you may open and review the various stages of the step in Clarity LIMS (see the *Review Run Data* on page 794 section).

Automated - NovaSeq Run (NovaSeq 6000 v3.0.1) contains two automations:

- Set Next Steps
- Update Lane Number

Set Next Steps automation is disabled (Set to Not Used). By default, the Sequencer API is used to set the next step for output samples.

The PCWP includes a Set Next Steps automation configured on the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0.1) step. Use this automation to override the default next step behavior. See the NovaSeq Integration v3.2.1 User and Configuration Guide for details.

Update Lane Number automation is triggered automatically on entry to the Record Details screen.

Do not disable or modify this automation to make sure that the Lane Number is displayed properly.

Review Run Data

Read summary metrics are recorded for the library pool. After the run is complete, open the step and review these metrics on the Next Steps screen in the Step Data section and the Sample Details table.

Step Data Section

Values should be populated in the following step UDFs/master step fields:

- Run ID
- Run Status
- Output Folder
- Current Cycle
- Current Read
- Loading Workflow Type
- Flow Cell ID
- Flow Cell Side
- Flow Cell Mode
- Flow Cell Part Number*
- Flow Cell Lot Number
- Flow Cell Expiration Date
- Instrument ID
- Instrument Type
- Instrument Control Software Version
- Firmware Version
- RTA Version
- Sequencing Log

Sample Details Table

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Summary metrics should be populated in the following UDFs/global fields. Values are aggregated across all lanes. Some values—for example Yield PF (Gb) R1—are summed, while others are averaged:

- Yield PF (Gb) R1
- Yield PF (Gb) R2*
- Reads PF (M) R1*
- Reads PF (M) R2*
- Cluster Density (K/mm^2) R1
- Cluster Density (K/mm^2) R2
- %PF R1
- %PF R2
- % Bases >=Q30 R1
- % Bases >=Q30 R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- % Aligned R1
- % Aligned R2
- % Error Rate R1
- % Error Rate R2

* For R2 Yield, R2 Error rate, and R2 Q30, there are discrepancies between the values shown in the Illumina SAV software and in the payload sent from the NVCS to Clarity LIMS. This issue is fixed in the upcoming NVCS release.

How the Integration Works

The following steps summarize how the Sequencer API integration works. For more detailed information, see the NovaSeq Integration v3.2.1 User and Configuration Guide.

1. When a run on the NovaSeq instrument is set up, NVCS sends a request for the run recipe.
    a. The Sequencer API validates that samples and containers are correctly queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0.1) step.

   b. Clarity LIMS sends a JSON response to NVCS. The response includes both the run recipe information and a link that can be used to download the sample sheet that is used with analysis software such as bcl2fastq2.
2. When the run starts, NVCS sends a RunStarted run status request. The Sequencer API then:
   - Validates reagent kit information in the RunStarted request:
     - Checks that the reagent kit exists. If it does not, creates it and enables it on the master step.
     - Checks that the reagent kit is activated. If it is not, activates it.
   - Starts the step for the queued samples. The step produces one output file placeholder per lane of the flow cell in use, based on the flow cell type.
   - Records all relevant information from the RunStarted request on the step, such as reagent lots and step fields.
3. At the end of the run, NVCS sends one of the following run status requests:
   - RunCompletedSuccessfully
   - RunEndedByUSer
   - RunErroredOut.
4. The Sequencer API then:
   - Updates the step with any new information. Currently, this update includes only the status and cycle/read information.
   - Updates the step with any new information. Currently, this update includes only the status and cycle/read information.
5. After primary analysis completes, NVCS sends a request containing the parsed run metrics. NVCS sends this request only when the run is completed successfully, but the API does not make this assumption and accepts the request regardless of status.
6. The Sequencer API then:
   - Records the metrics into the fields on file placeholder outputs in Clarity LIMS.
   - Completes the step in Clarity LIMS if the status is RunCompletedSuccessfully. For all other status options, the step remains in progress. This process is the default autocomplete step behavior. For other configuration options, see the NovaSeq Integration v3.2.1 User and Configuration Guide.

The NovaSeq Integration v3.2.1 User and Configuration Guide provides detailed information on how the following components work with the Sequencer API integration:

- Sample sheet generation and contents
- NovaSeq run
- User authentication and login
- Run recipe contents

Troubleshooting

If an automation trigger does not appear to run its corresponding scripts, see the following articles:

- *Troubleshooting Automated Informatics* section in the Clarity LIMS documentation.
- *Troubleshooting EPP/Automation* in the API > Clarity LIMS API and Database > Advanced Topics section

If an error occurs that does not provide direction on how to proceed, complete the following steps:

1. Confirm the version of the installed Illumina NovaSeq Integration Package by running the following command on the Clarity LIMS server command line:

```
rpm -qa | grep -i novaseq
```

2. If the error is related to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0.1) step, review the log file information. See *Logging* on page 797.

If the automated run step starts but does not complete, review the following information:

1. Use one of the following methods to open the in progress step in Clarity LIMS:
   - Log in to the default user account.
   - In Lab View, find the step in the Recent Activities pane.
   - Search for the step in Clarity LIMS using either the Library Tube or Flow Cell barcode as the search term, depending on whether this run is a NovaSeq Standard or NovaSeq Xp run.
2. On the Record Details screen, the Sequencing Log multiline text field contains logging information.
   - If you are unable to reach the Record Details screen, or if the Sequencing Log field does not contain enough information to resolve the issue, review the `sequencer-api.log` file described in the following section.
3. Contact the Clarity LIMS support team, supplying the relevant information from the troubleshooting steps already performed.

Additional troubleshooting information is provided on the Illumina Integrations FAQ page.

Logging

In addition to updating the Sequencing Log multiline text field on the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0.1) step, the Sequencer API writes a detailed log file to `/opt/gls/clarity/tomcat/current/logs/sequencer-api.log`.

Log messages include the Library Tube ID and Flow Cell ID whenever the messages are related to a sequencing run-related request. In Clarity LIMS workflows, these IDs are recorded as container names.

Some log messages are not directly related to a sequencing run request, such as downloading the sample sheet. In this case, the file LIMS ID and file name are included in log messages. The sample sheet downloaded by the instrument, by default, has a name that includes the ID (Library Tube for Standard or Flow Cell for Xp) of the sequencing container. The relevant container name search finds most of the log messages for this request as well. However, to find all messages for downloading a file,

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you must know the LIMS ID of the file. Find this information by looking in the Clarity LIMS API and, for sample sheets used in a run, in the recipe response. The recipe response can also be found in the log file.

The following example lines are from the log file:

```
2018-06-19 21:32:19.705 INFO --- [] SequencerAPIApplication : Started
SequencerAPIApplication in 7.026 seconds (JVM running for 15.964)
...
2018-06-19 21:34:44.564 INFO --- [Library Tube Id=NV1234567-LIB, Flow Cell
Id=RM123DSXX] LimsApiLookupUtil : Searching for containers with name 'NV1234567-
LIB' or 'RM123DSXX'
....
2018-06-19 21:35:27.158 INFO --- [] FileService : Successfully retrieved file
'NV1234567-LIB.csv' with LIMS ID 40-51 through the API.
```

NovaSeq 6000 Integration v3.2.2 Release Notes

Compatibility

- Clarity LIMS v4.2 to v5.3. Not compatible with v5.4 or later.
- NGS Extensions Package v5.19. Not compatible with v5.20 or later.
- Preconfigured Workflow Package (PCWP) v1.2.2. Not compatible with v2.0 or later.
- NovaSeq Control Software (NVCS) v1.7.0.
- Illumina Bcl2FastQ v2.20.

Features to Note for this Release

This release of Clarity LIMS integration package provides:

- Support for the NovaSeq Control Software v1.7.0 with v1.5 reagents.
- Support for reverse complement workflow in sample sheet generation.
- Support for Unique Molecular Identifiers (UMI) configuration that is populated in sample sheet.
- Updated workflow (NovaSeq 6000 v3.0.1) delivered directly from PCWP v1.2.2.

Defect Fixed

- Fixed lane number discrepancy issue in Automated - NovaSeq Run step.

NovaSeq 6000 Integration v3.2.1 Configuration and User Interaction

The Illumina NovaSeq 6000 Integration Package v3.2.1 supports the integration of BaseSpace Clarity LIMS to Illumina NovaSeq 6000 sequencers.

Note the following key changes for this version of the integration:

- A move from a file-based to an API-based integration using the Sequencer API.
- NovaSeq-specific configuration is now installed as part of the Preconfigured Workflows Package (PCWP) v1.2. This configuration includes new template path locations and sample sheet generation with Template File Generator (TFG).

Configuration changes in v3.2.1 include the following:

- Support for Sequencer API
- Support for SPrime (SP) flow cell
- Support for Long-Read sequencing (SP flow cell only)
- Support for asymmetric read lengths

This *User and Configuration Guide* describes the integration and includes information about the preconfigured workflows, protocols, steps, and automations. The guide also lists installed components, and provides configuration requirements, rules, and constraints.

For instructions on validating and troubleshooting the Illumina NovaSeq 6000 Integration, see the *NovaSeq 6000 Integration v3.2.1 Validation and Troubleshooting* on page 875.

The configuration provided in this integration has been established to support NovaSeq 6000 lab processes. Any configuration changes to protocols or workflows - including renaming protocols, steps, and fields - could break process.

Overview

The integration includes the following:

- Preconfigured NovaSeq 6000 v3.0 workflow that maps to lab protocols and instrument runs.
- Preconfigured protocols:
  - Run Format (NovaSeq 6000 v3.0)
  - NovaSeq Standard (NovaSeq 6000 v3.0) (supports the loading of pooled libraries into a library tube)
  - NovaSeq Xp (NovaSeq 6000 v3.0) (supports individual lane loading on the NovaSeq)
  - AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0)
- Automated generation of a sample sheet for use with bcl2fastq2 v2.20 analysis software. This file is automatically uploaded to the sequencing instrument via the Sequencer API and is used to set up the run.
- Automatic validation of run setup information. This information is used to create the run recipe and initiate the run.
- Automated tracking of the NovaSeq sequencing run and parsing of run statistics into the LIMS, including:
  - Progress and metrics of sequencing run
  - Per-instrument sequencing runs (tracked as part of the Run ID field value)

  - Sequencing run parameters
  - Sequencing run data directory location
  - Real Time Analysis v3 (RTA3) version and other run specific information
- [Optional] Preconfigured Library Prep Validation v1.0 workflow used for validation purposes only. The workflow contains a single-step protocol that models the library prep required to produce normalized libraries that are ready for the NovaSeq 6000 v3.0 workflow. See the *NovaSeq 6000 Integration v3.2.1 Validation and Troubleshooting* on page 875.

Information on these features is provided in the following sections.

Prerequisites and Assumptions

It is assumed that samples enter the NovaSeq 6000 v3.0 workflow as normalized libraries. That is, before they are assigned to the workflow:

- Samples have been accessioned into the LIMS.
- Samples have been run through QC and library prep.
- Samples have been normalized, and the value is captured in a field called Normalized Molarity (nM).

For details on sample accessioning, see the following articles in the Samples section of the LIMS documentation for your version: LIMS Documentation v5.0 and v5.1 or LIMS Documentation v4.1 and v4.2:

- Sample Accessioning
- Using Sample Lists to Upload and Modify Samples

Samples can be assigned to the NovaSeq 6000 v3.0 workflow automatically (using a routing script), or manually from the Projects and Samples dashboard (see the *Assigning Samples to Workflows* article in the LIMS Documentation).

Workflows, Protocols, and Steps

The Illumina NovaSeq 6000 Integration Package v3.2.1 includes two workflows:

- NovaSeq 6000 v3.0
- Library Prep Validation v1.0 (optional, but recommended for validation purposes)

The following table lists and describes the protocols and steps included in these workflows.

Library Prep Validation v1.0 workflow

Protocol name	Protocol purpose	Steps included in protocol
Library Prep Validation v1.0	Included for validation purposes only, this protocol models the library prep steps required to advance samples to the Run Format (NovaSeq 6000 v3.0) protocol. The protocol contains a single step - Library Prep Validation v1.0. At the end of this step, a routing script sends the samples to the first step of the NovaSeq 6000 v3.0 workflow - Define Run Format (NovaSeq 6000 v3.0).	1. Library Prep Validation v1.0

NovaSeq 6000 v3.0 workflow

Protocol name	Protocol purpose	Steps included in protocol
Run Format (NovaSeq 6000 v3.0)	Allows for the assignment of per sample values for Loading Workflow Type, Normalized Molarity, Flowcell Type, and Final Loading Concentration (pM): • Loading Workflow Type: User selects from NovaSeq Standard or NovaSeq Xp. • Normalized Molarity: User enters a value for each sample. • Flowcell Type: User selects from options SP, S1, S2, or S4. • Final Loading Concentration (pM): User selects from options 225 (PCR-free workflows) or400 (Nano workflows), or may enter a different value. Compares each sample's Normalized Molarity value with the Minimum Molarity value. Routing script sends samples to the NovaSeq Standard or NovaSeq Xp protocol, according to the selected Loading Workflow Type. Samples with Normalized Molarity less than Minimum Molarity are removed from the workflow.	1. Define Run Format (NovaSeq 6000 v3.0)

Library Prep Validation v1.0 workflow

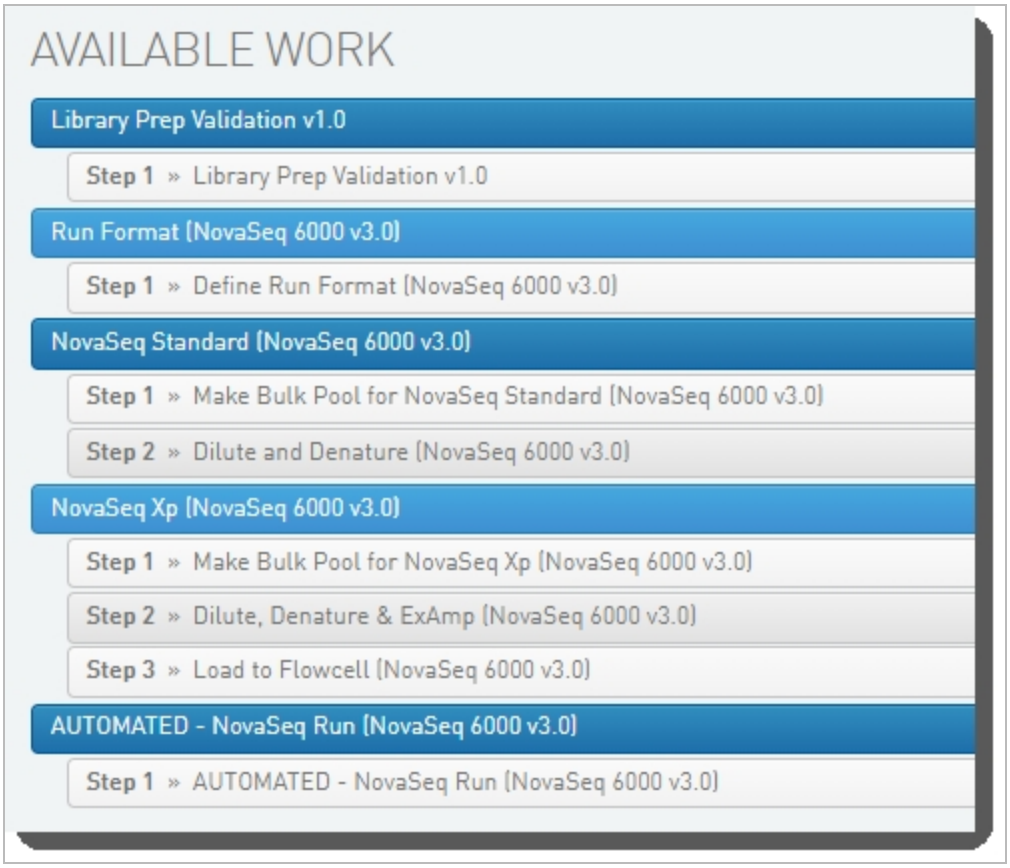
Protocol name	Protocol purpose	Steps included in protocol

NovaSeq 6000 v3.0 workflow

Protocol name	Protocol purpose	Steps included in protocol
NovaSeq Standard (NovaSeq 6000 v3.0)	Samples are pooled and added to the library tube in preparation for the NovaSeq run. Run setup information is validated and sample sheet is generated. Routing script sends library tube to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v30) protocol.	1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.0) 2. Dilute and Denature (NovaSeq 6000 v3.0)
NovaSeq Xp (NovaSeq 6000 v3.0)	Samples are pooled and added to lanes on the NovaSeq flow cell. The flow cell type is determined by the option selected in the Define Run Format (NovaSeq 6000 v3.0) step. Run setup information is validated and sample sheet is generated. Routing script sends flow cell to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v30) protocol.	1. Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.0) 2. Dilute, Denature and ExAmp (NovaSeq 6000 v3.0) 3. Load to Flowcell (NovaSeq 6000 v3.0)
AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0)	All samples complete the workflow by going through this protocol. This protocol contains one fully-automated step. **!** The user must not add samples to the Ice Bucket or start the step. The integration will do this automatically.	1. AUTOMATED - NovaSeq Run (NovaSeq 6000 v30)

The following image show the protocols and their steps displayed in the LIMS Lab View.

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Validation Workflow

The Library Prep Validation v1.0 workflow allows for validation of the system after installation is complete. For details, see the *NovaSeq 6000 Integration v3.2.1 Validation and Troubleshooting* on page 875.

Step Automations

The following table lists the step automations included in the NovaSeq 6000 v3.0 workflow. Automation details are provided in the sections following the table.

For details on the validation workflow, see the *NovaSeq 6000 Integration v3.2.1 Validation and Troubleshooting* on page 875.

Protocol	Step	Automation
1. Run Format (NovaSeq 6000 v3.0)	1. Define Run Format (NovaSeq 6000 v3.0)	• Routing Script • Set Next Steps

Protocol	Step	Automation
2. NovaSeq Standard (NovaSeq 6000 v3.0)	1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.0)	• Calculate Volumes • Set Next Step • Validate Inputs Flowcell Type and Single Pool • Validate Unique Indexes *Not used. This functionality is handled by the core LIMS configuration for pooling.*
	2. Dilute and Denature (NovaSeq 6000 v3.0)	• Routing Script • Validate Library Tube Barcode • Validate Run Setup and Generate Sample Sheet • Validate Single Input
3. NovaSeq Xp (NovaSeq 6000 v3.0)	1. Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.0)	• Calculate Volumes • Set Next Step • Validate Inputs Flowcell Type and Single Pool • Validate Unique Indexes *Not used. This functionality is handled by the core LIMS configuration for pooling.*
	2. Dilute, Denature and ExAmp (NovaSeq 6000 v3.0)	• Calculate Volumes • Set Next Step • Validate Inputs Flowcell Type
	3. Load to Flowcell (NovaSeq 6000 v3.0)	• Validate Flowcell Barcode • Validate Inputs and Selected Container • Validate Run Setup and Generate Sample Sheet
4. AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0)	1. AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0)	• Set Next Steps *Not used. By default, this functionality is determined by the Sequencer API.If required, you can enable the automation and use it to override the next step behavior used by the Sequencer API.*

Configuration Details

The sections below describe the configuration installed with the Illumina NovaSeq 6000 Integration.

As of LIMS v5, the term user-defined field (UDF) has been replaced with custom field in the user interface (the API resource is still called udf). There are two types of custom fields:

- Master step fields - configured on master steps. These fields only apply to the master step on which they are configured, and the steps derived from those master steps.
- Global fields - configured on entities such as submitted sample, derived sample, measurement, etc. These fields apply to the whole LIMS system.

Protocol 1: Run Format (NovaSeq 6000 v3.0

This protocol sets the Loading Workflow Type and allows the user to choose the appropriate Flowcell Type and Final Loading Concentration (pM). At the end of the protocol, a routing script sends the normalized libraries to either the NovaSeq Standard (NovaSeq 6000 v3.0) or the NovaSeq Xp (NovaSeq 6000 v3.0) protocol.

This protocol contains one step: Define Run Format (NovaSeq 6000 v3.0), described below.

Step 1. Define Run Format (NovaSeq 6000 v3.0)

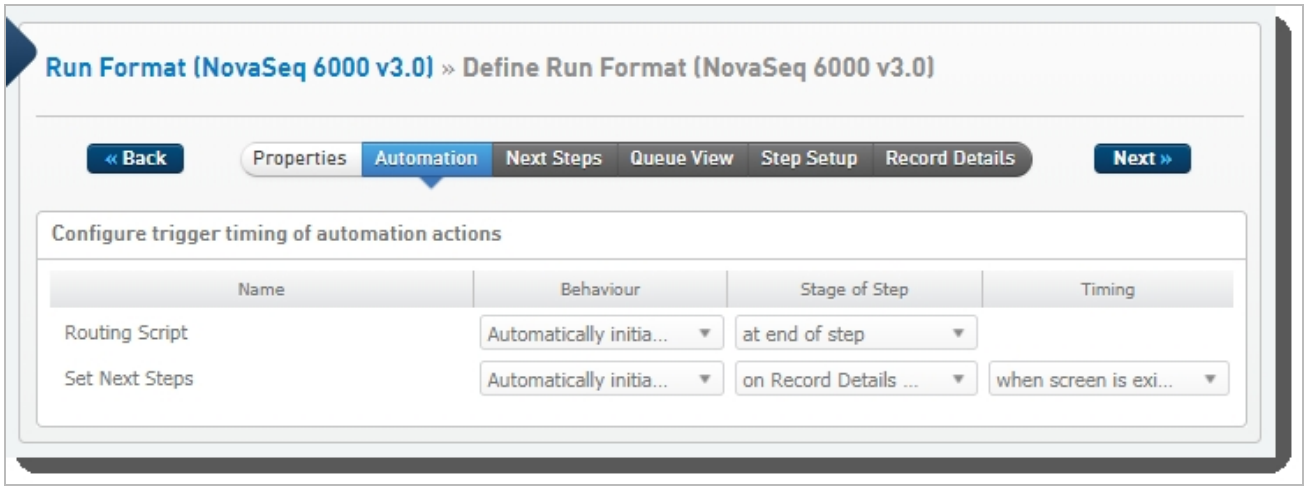
Step input: NTP (normalized libraries)

Step output: None

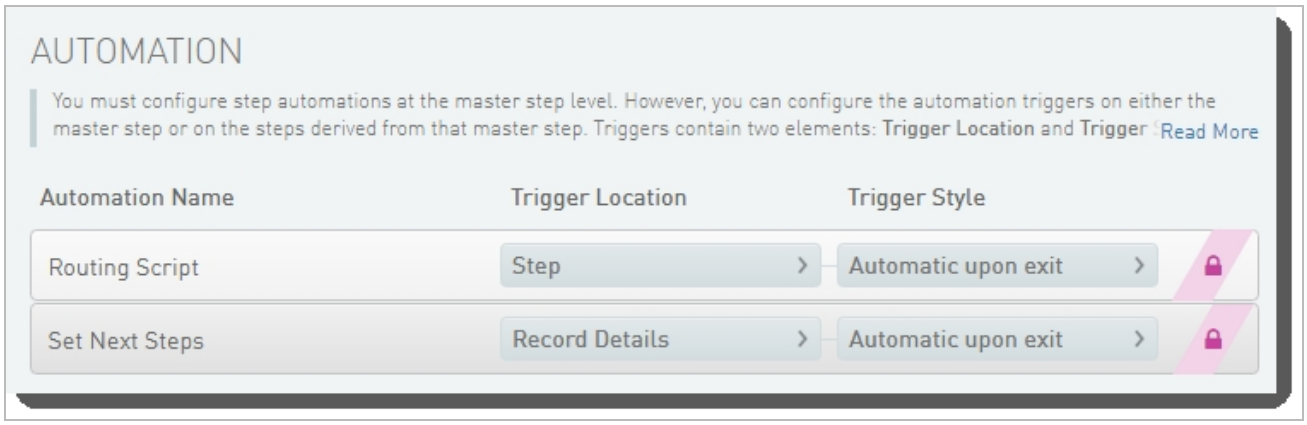
The following automations are configured on this step:

- Routing Script
- Set Next Steps

Define Run Format (NovaSeq 6000 v3.0) automation configuration - LIMS v4.2.x:



Define Run Format (NovaSeq 6000 v3.0) automation configuration - LIMS v5.x:



These automations are described below, in the order in which they are triggered at run time.

Set Next Steps

Automatically triggered on exit of the Record Details screen, this automation does the following:

- Sets the next step for samples to REMOVE:

```
nextStep = ::REMOVE::
```

- Calculates the Minimum Molarity using the following formula:

```
input.::Minimum Molarity (nM):: = (5 * input.::Final Loading Concentration
(pM)::)/1000
```

- Checks Normalized Molarity value. For samples with no Normalized Molarity value (i.e., empty value, not including 0), generates an error message informing the user that the field cannot be empty:

```
if (!input.hasValue(::Normalized Molarity (nM)::)) { \
  fail(::The Normalized Molarity cannot be empty.::) ;
}
```

- Compares each sample's Normalized Molarity value with the Minimum Molarity value. If Normalized Molarity value is lower than the Minimum Molarity value, sets the sample's Loading Workflow Type to [Remove from workflow] and records a message in the Warning field for the sample:

```
else if (input.::Normalized Molarity (nM):: < input.::Minimum Molarity (nM)::)
{ \
  input.::Warning:: = ::The Normalized Molarity is too low.:: ; \
  input.::Loading Workflow Type:: = ::[Remove from workflow]:: ; } \
else { input.::Warning:: = ::n/a:: }
```

  At this point the user has two options:

  - Correct the Normalized Molarity value on the Record Details screen. User will also need to edit the Loading Workflow Type field and set it to NovaSeq Standard or NovaSeq Xp, as applicable.

– Complete the protocol without correcting the Normalized Molarity value. In this case, those samples will be removed from the workflow.

Routing Script

Automatically triggered on exit of the step, this automation invokes the changeWorkflow script, which routes step inputs appropriately.

- Samples with Loading Workflow Type field value = NovaSeq Standard are routed to the NovaSeq 6000 v3.0 workflow and queued for the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.0) step.
- Samples with Loading Workflow Type field value = NovaSeq Xp are routed to the NovaSeq 6000 v3.0 workflow and queued for the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.0) step.

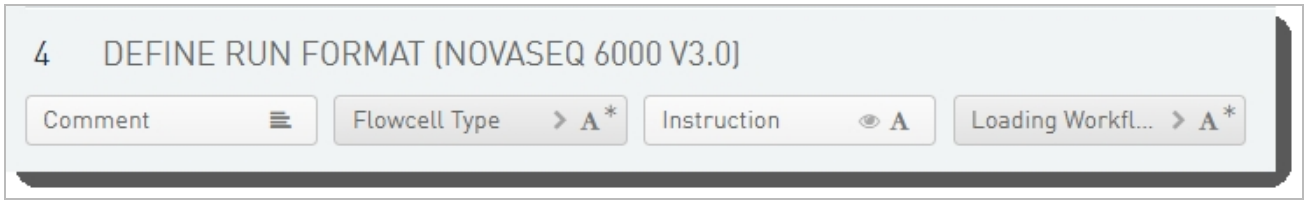
Default automation command line:

```
bash -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -u {username} -p {password} -i {stepURI:v2} -l
{compoundOutputFileLuid0} script:changeWorkflow \\
--FIELD_NAME 'Loading Workflow Type' \
--FIELD_VALUE 'NovaSeq Standard' \
--WORKFLOW 'NovaSeq 6000 v3.0' \
--STEP 'Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.0)' \
--INPUTS_OR_OUTPUTS 'INPUTS' \\
--FIELD_NAME 'Loading Workflow Type' \
--FIELD_VALUE 'NovaSeq Xp' \--WORKFLOW 'NovaSeq 6000 v3.0' \
--STEP 'Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.0)' \
--INPUTS_OR_OUTPUTS 'INPUTS'"
```

Step UDFs/Custom Fields

There are four fields defined on the Define Run Format (NovaSeq 6000 v3.0) step.

These are shown below in the BaseSpace Clarity LIMS v5.x Configuration area (Custom Fields > Master Step Fields tab).



See the following table for LIMS v4.2.x and LIMS v5.x field configuration details:

Field Name	Field Type	Field Constraints	Preset Values/Additional Options and Dropdown Items
Comment	Multi-line Text	• Required: No • Read Only: No	None
Flowcell Type	Single-line Text (v4.2.x) Text Dropdown (v5.x)	• Required: Yes • Read Only: No • Custom Entries: Yes	• SP • S1 • S2 • S4
Instruction	Single-line Text (v4.2.x) Text (v5.x)	• Required: No • Read Only: Yes	• Default: Add Flowcell Type and Loading Workflow Type below
Loading Workflow Type	Single-line Text (v4.2.x) Text Dropdown (v5.x)	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp

Global UDFs/Custom Fields

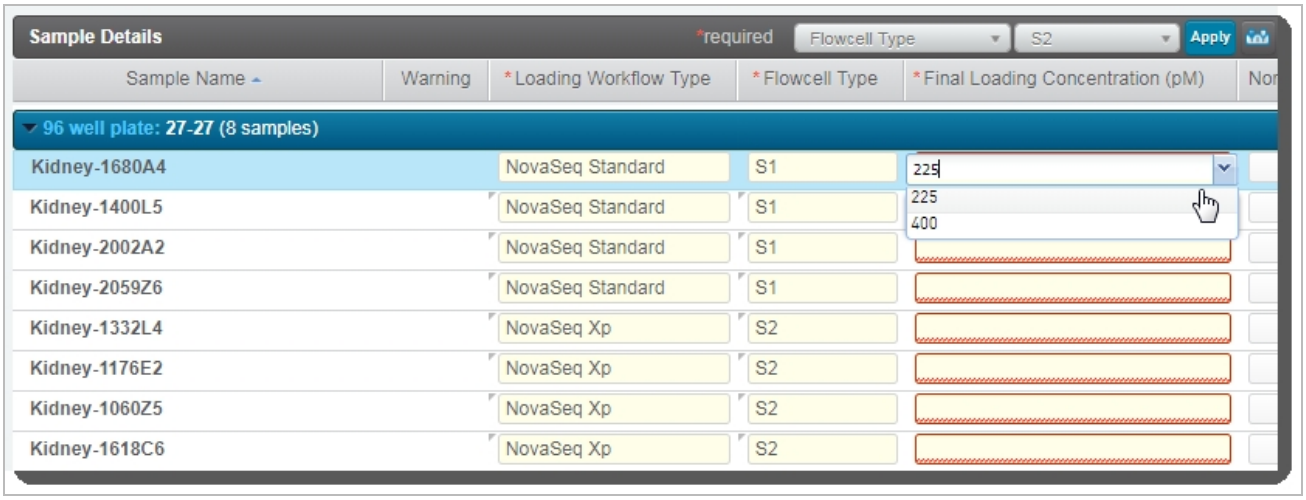
The following table lists the global UDFs/custom fields that are configured to display on the Define Run Format (NovaSeq 6000 v3.0) step.

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Configured on Analyte in LIMS v4.2.x and Derived Samplein LIMS v5.x:			
Adjusted Per Sample Volume (ul)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Final Loading Concentration (pM)	Numeric (v4.2.x) Numeric Dropdown (v5.x)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 225 • 400 • Decimal Places Displayed: 0
Flowcell Type	Single-line Text (v4.2.x) Text Dropdown (v5.x)	• Required: Yes • Read Only: No • Custom Entries: No	• SP • S1 • S2 • S4
Loading Workflow Type	Single-line Text (v4.2.x) Text Dropdown (v5.x)	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Minimum Molarity (nM)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2
Normalized Molarity (nM)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2
Per Sample Volume (ul)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Warning	Single-line Text (v4.2.x) Text Dropdown (v5.x)	• Required: No • Read Only: Yes • Custom Entries: Yes	• The Normalized Molarity (nM) is too low. • n/a

User Interaction

1. On the Record Details screen, the Sample Details table contains the following fields that must be completed (values can vary across samples):
    - Loading Workflow Type: Users selects NovaSeq Standard or NovaSeq Xp from the drop-down list.
    - Flowcell Type: User selects SP, S1, S2, or S4 from the drop-down list.
    - Final Loading Concentration (pM): User selects from the two preset options - 225 (for PCR-free workflows) or400 (for Nano workflows), or may enter a different value.
    - Normalized Molarity (nM):These values are copied over from the previous step. If the user did not populate this column during library prep, they can enter the values here.



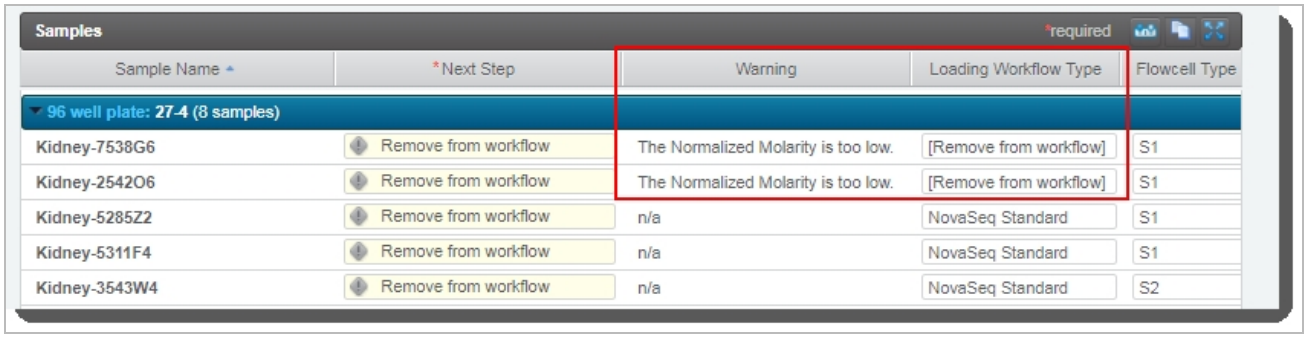
- User clicks Next Steps. This triggers the Next Steps automation, which does the following:

- Sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value, and requires it in order to successfully advance samples to the next step.
- Calculates the Minimum Molarity.
- Checks Normalized Molarity value. For samples with no Normalized Molarity value (i.e., empty value, not including 0), generates an error message informing the user that the field cannot be empty.
- Compares each sample's Normalized Molarity value with the Minimum Molarity value.

2. On the Assign Next Steps screen:
   - In the Sample Details table, the Next Step for all samples is pre-populated with Remove from workflow - regardless of the Loading Workflow Type.

> The user must not change this value. If Next Step is not set to Remove from workflow, the routing script will not be able to route samples correctly.

For samples whose Normalized Molarity value was found to be lower than the Minimum Molarity value, the Loading Workflow Type is set to Remove from workflow and a message is recorded in the Warningfield for the sample:



At this point the user has two options:

- Return to the Record Details screen and adjust the Normalized Molarity value so that it equals or exceeds the Minimum Molarity value. User will also need to set the Loading Workflow Type to NovaSeq Standard.
- Complete the protocol without correcting the Normalized Molarity value. In this case, the samples in question will be removed from the LIMS workflow.

3. User clicks Finish Step. The Routing Script automation is triggered:
   - Samples whose Loading Workflow Type is set to Remove from workflow (i.e., whereNormalized Molarity value is lower than the Minimum Molarity) are removed from the LIMS workflow.
   - Samples whose Loading Workflow Type is set to NovaSeq Standard are routed to the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.0) step. This is the first of two steps in the NovaSeq Standard (NovaSeq 6000 v3.0) protocol.

- Samples whose Loading Workflow Type is set to NovaSeq Xp are routed to the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.0) step. This is the first of three steps in the NovaSeq Xp (NovaSeq 6000 v3.0) protocol.

Protocol 2: NovaSeq Standard (NovaSeq 6000 v3.0)

Samples are routed to this protocol if their Loading Workflow Type value is set to NovaSeq Standard. Samples are pooled and added to a library tube in preparation for the NovaSeq run.

At the end of this protocol, a routing script sends the library tube to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0) protocol.

This protocol contains two steps, described below:

- Step 1: Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.0)
- Step 2: Dilute and Denature (NovaSeq 6000 v3.0)

Step 1: Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.0)

In this step, user manually places libraries into a single pool. Resuspension buffer and reagents are added.

User must only create only one pool per step.

Step input: NTP (normalized libraries)

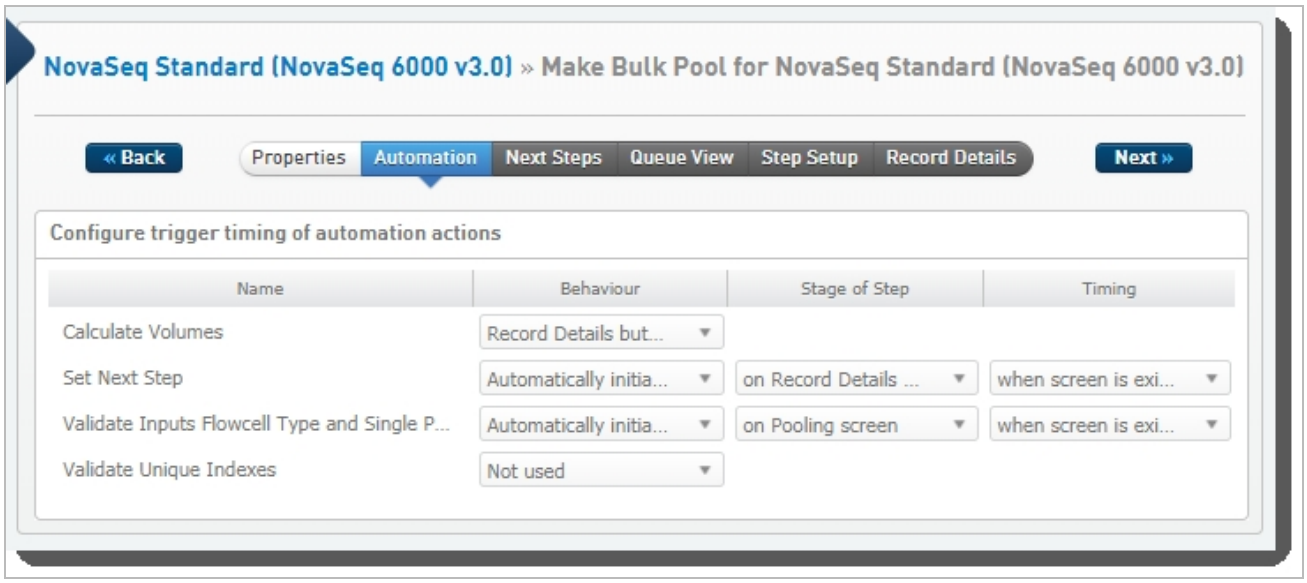
Step output: Bulk pool

The following automations are configured on the step:

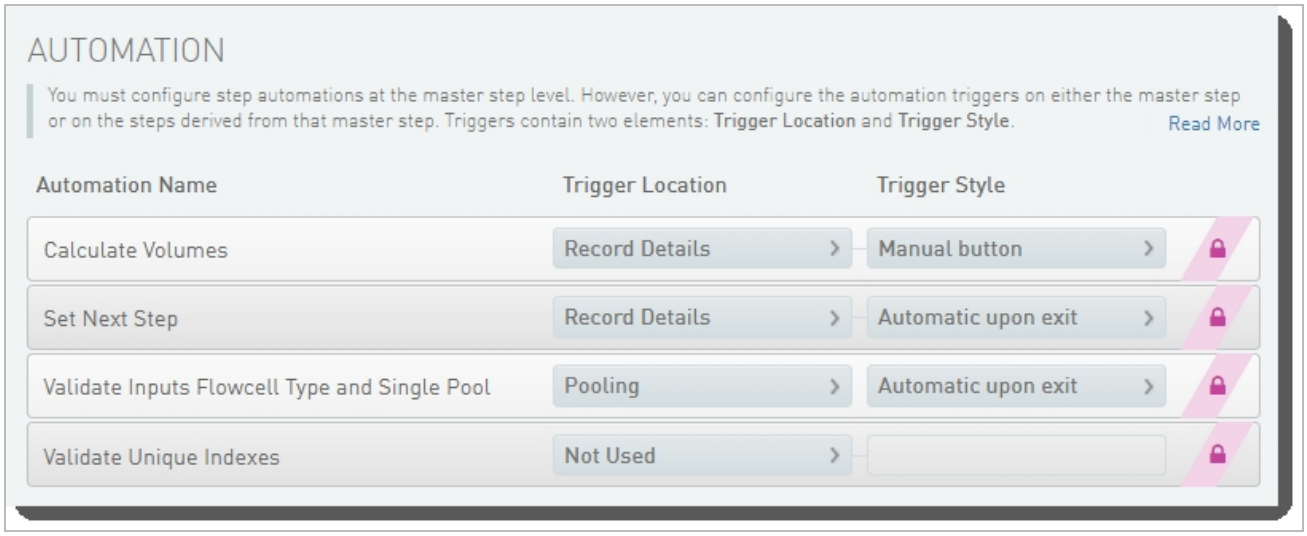
- Calculate Volumes
- Set Next Step
- Validate Inputs Flowcell Type and Single Pool
- Validate Unique Indexes*(Not used. This functionality is handled by the core LIMS configuration for pooling.)*

Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.0) automation configuration - LIMS v4.2.x:

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Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.0) automation configuration - LIMS v5.x:



These automations are described below, in the order in which they are triggered at run time.

Validate Inputs Flowcell Type and Single Pool

Automatically triggered on exit of the Pooling screen, this automation:

- Checks that all samples in the pool have the same Flowcell Type assigned to them.
- Checks that only one pool has been created. Changes the value of error and logging messages to reference the type of pool passed as 'bulk' pool.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar \script:validate_flowcell_for_input_pools \
  -i {stepURI:v2} \
  -u {username} \
```

```
  -p {password} \
  -l {compoundOutputFileLuid1} \
  -validateSingleOutput true \
  -poolType bulk"
```

Calculate Volumes

Automatically triggered when the user clicks the Calculate Volumes button on the Record Details screen, this automation:

- Calculates the number of samples in the pool:

```
-exp 'step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: +
1'
```

- Sets the value of the Bulk Pool Volume (ul) field, based on the selected Flowcell Type:

```
-exp 'if ( input.::Flowcell Type:: == ::SP:: ) {
  step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 100
+ 30
} ;

if ( input.::Flowcell Type:: == ::S1:: ) {
  step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 100
+ 30
} ;
if ( input.::Flowcell Type:: == ::S2:: ) {
  step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 150
+ 30
} ;
if ( input.::Flowcell Type:: == ::S4:: ) {
  step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 310
+ 30
} ;
```

- Calculates the Per Sample Volume (ul) to be added to the pool:

```
input.::Per Sample Volume (ul):: = ( ( (input.::Final Loading Concentration
(pM):: * 5 / 1000) / input.::Normalized Molarity (nM):: ) * step.::Bulk Pool
Volume (ul):: ) / step.::Number of Samples in Pool::
```


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To ensure accurate pipetting of each sample in a pool for sequencing, the Per Sample Volume (ul) value must be equal to or higher than the Minimum Per Sample Volume (ul) (default value set at 5, may be edited by the user).

Assuming the default Minimum Per Sample Volume (ul) value of 5, for a given batch:

1. If the smallest Per Sample Volume (ul) value is less than 5, the LIMS automatically assigns a value of 5 to the sample's Adjusted Per Sample Volume (ul) field.
2. The LIMS then adjusts the Adjusted Per Sample Volume (ul) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

- Calculates the Total Sample Volume (ul) field value:

```
-exp 'step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: +
input.::Adjusted Per Sample Volume (ul)::'
```

- If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (ul) field value:

```
if (step.::Total Sample Volume (ul):: >= step.::Bulk Pool Volume (ul)::)
{output.::RSB Volume (ul):: = 0} else {output.::RSB Volume (ul):: = step.::Bulk
Pool Volume (ul):: - step.::Total Sample Volume (ul)::} ;
```

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ;output.::Loading Workflow
Type:: = input.::Loading Workflow Type:: ;
```

- Sets the Volume of Pool to Denature (ul) value and calculates NaOH Volume (ul) and Tris-HCl Volume (ul) values, based on the Flowcell Type:

```
if ( input.::Flowcell Type:: == ::SP:: ) { output.::Volume of Pool to Denature
(ul):: = 100 ; output.::NaOH Volume (ul):: = 25 ; output.::Tris-HCl Volume (ul)::
= 25 } ;
if ( input.::Flowcell Type:: == ::S1:: ) { output.::Volume of Pool to Denature
(ul):: = 100 ; output.::NaOH Volume (ul):: = 25 ; output.::Tris-HCl Volume (ul)::
= 25 } ;
if ( input.::Flowcell Type:: == ::S2:: ) {output.::Volume of Pool to Denature
(ul):: = 150 ; output.::NaOH Volume (ul):: = 37.50 ; output.::Tris-HCl Volume
(ul):: = 37.50 } ;
if ( input.::Flowcell Type:: == ::S4:: ) { output.::Volume of Pool to Denature
(ul):: = 310 ; output.::NaOH Volume (ul):: = 77.50 ; output.::Tris-HCl Volume
(ul):: = 77.50 }
```

- Uses the NovaSeq_Standard_Bulk_Pool1.csv, NovaSeq_Standard_Bulk_Pool2.csv and NovaSeq_Standard_Bulk_Pool3.csv template files to generate a single CSV file containing information about the pool and the samples it contains. The generated file is available for download by the user on the Step Setup screen of the following step - Dilute and Denature (NovaSeq 6000 v3.0).

```
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p {password}
-l {compoundOutputFileLuid1} \ script:driver_file_generator \
  -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Standard_Bulk_
Pool1.csv \
  -o 1.csv \ script:driver_file_generator \
  -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Standard_Bulk_
Pool2.csv \
  -o 2.csv \ script:driver_file_generator \
  -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Standard_Bulk_
Pool3.csv \
  -o 3.csv \
&& cat 1.csv 2.csv 3.csv > {compoundOutputFileLuid0}.csv
```

Set Next Step

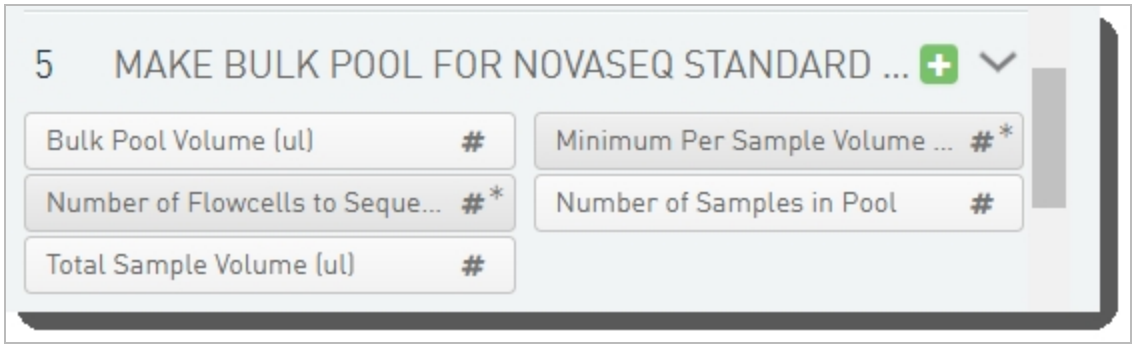
Automatically triggered on exit of the Record Details screen, this automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol - Dilute and Denature (NovaSeq 6000 v3.0):

```
nextStep = ::ADVANCE::
```

Step UDFs/Custom Fields

There are five fields defined on the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.0) step.

These are shown below in the BaseSpace Clarity LIMS v5.x Configuration area (C ustom Fields > Master Step Fields tab).



See the following table for LIMS v4.2.x and LIMS v5.x field configuration details:

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Bulk Pool Volume (ul) (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2
Number of Flowcells to Sequence	Numeric	• Required: Yes • Read Only: No	• Range From:1 to 10 • Decimal Places Displayed: 0
Total Sample Volume (ul) (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Default: 0 • Decimal Places Displayed: 2
Minimum Per Sample Volume (uL)	Numeric	• Required: Yes • Read Only: No	• Default: 5 • Decimal Places Displayed: 2
Number of Samples in Pool (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Default: 0 • Decimal Places Displayed: 0

Global UDFs/Custom Fields

The following table lists the global UDFs/custom fields that are configured to display on the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.0) step.

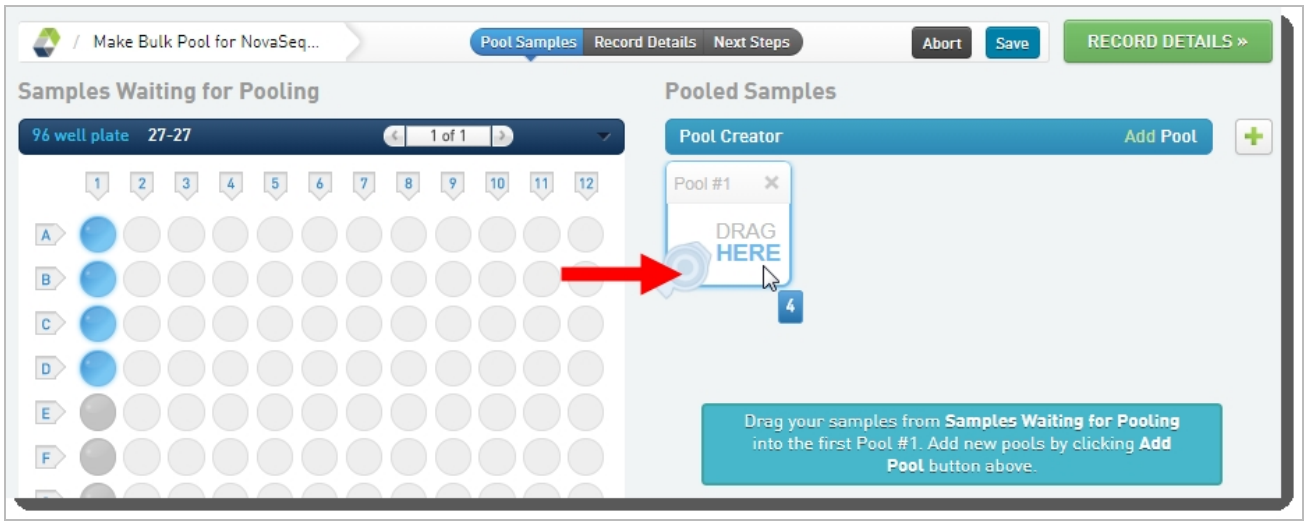
Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Configured on Analyte in LIMS v4.2.x and Derived Samplein LIMS v5.x:			
Flowcell Type	Single-line Text (v4.2.x) Text Dropdown (v5.x)	• Required: Yes • Read Only: No • Custom Entries: No	• SP • S1 • S2 • S4
Loading Workflow Type	Single-line Text (v4.2.x) Text Dropdown (v5.x)	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
NaOH Volume (ul)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
RSB Volume (ul)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Tris-HCl Volume (ul)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Volume of Pool to Denature (ul) *Used in Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.0) step only. Displays on Record Detailsscreen and in the generated CSV file.*	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0

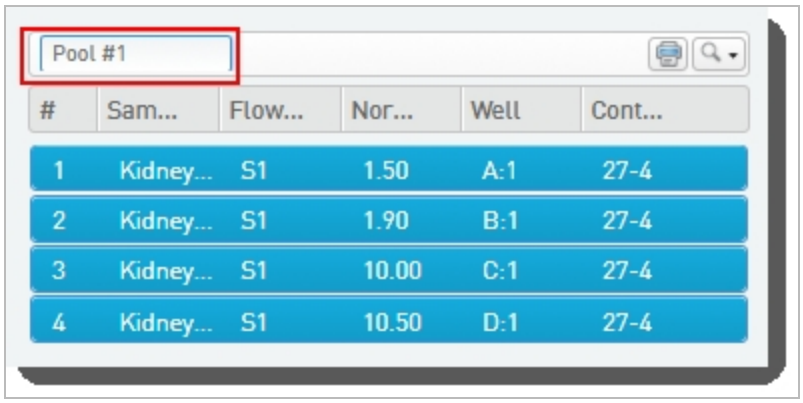
User Interaction

1. On the Queue screen, user adds samples of the same Flowcell Type to the Ice Bucket and clicks View Ice Bucket.
2. On the Ice Bucket screen, user clicks Begin Work.
3. On the Pooling screen, user:
    - Creates a pool by dragging samples into the Pool Creator.

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- Names the pool, or accepts the default name - Pool #1.

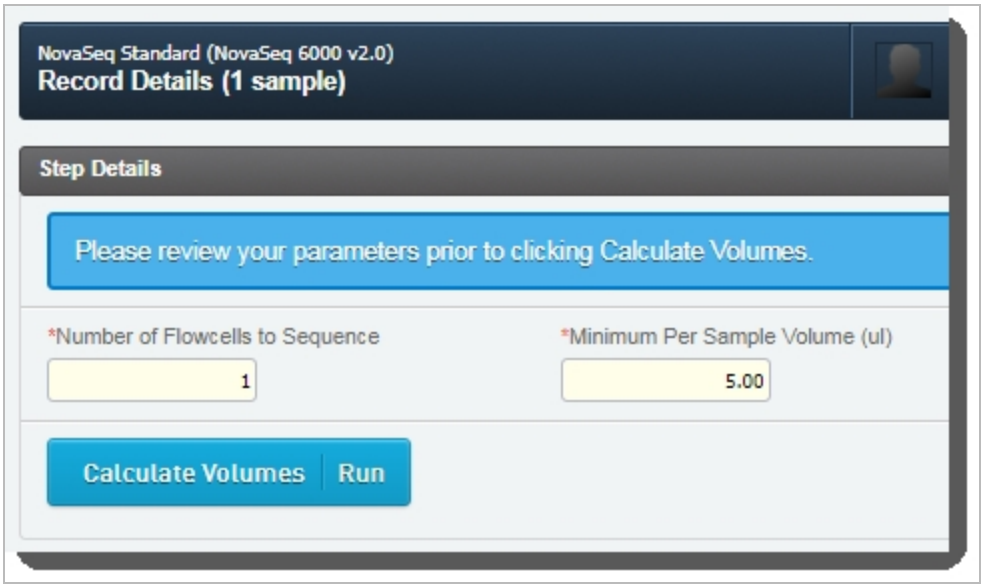


- Clicks Record Details.

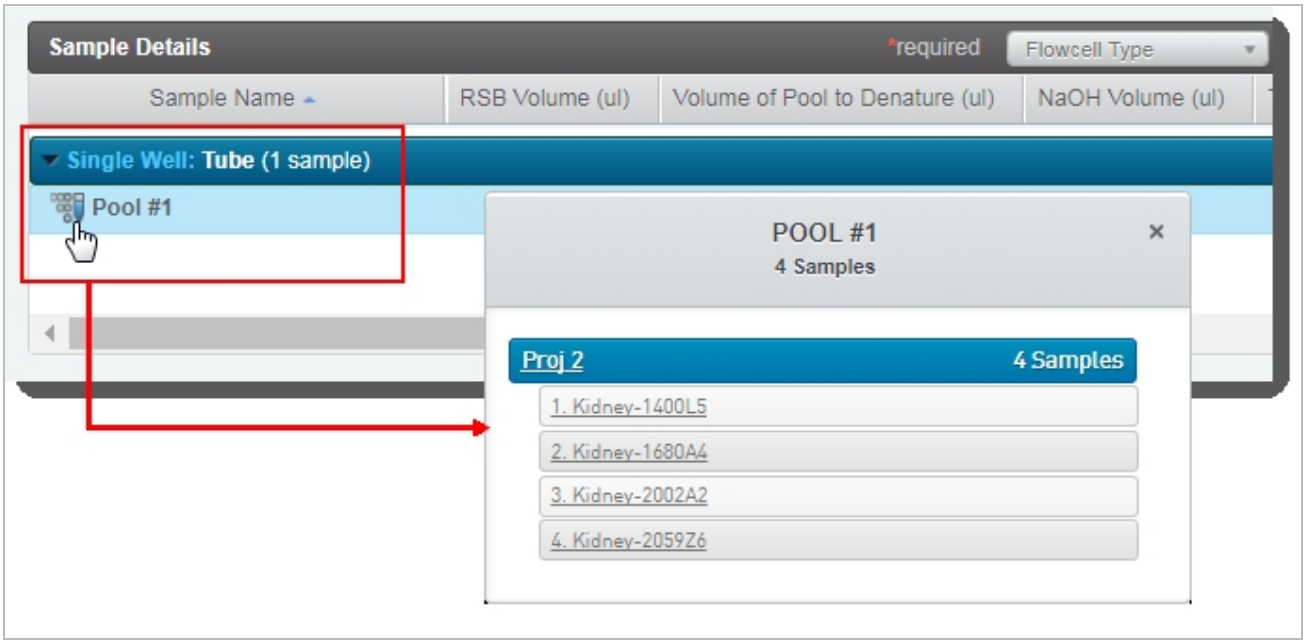
  On exit from the Pooling screen, the Validate Inputs Flowcell Type and Single Pool is triggered. The automation checks that all samples in the pool have been assigned the same Flowcell Type, and only one pool has been created.

4. On the Record Details screen, the Step Details area contains two required fields:
   a. Number of Flowcells to Sequence - The value entered in this field is used in volume calculations, to ensure that volumes are sufficient for the number of times the pool will be sequenced.
   b. Minimum Per Sample Volume (ul) - In many applications, and particularly where robotics are used, a minimum sample volume is required. The value in this field is used to calculate how much of each sample will be included in the pool. The field is pre-populated with the configured default value, 5 ul, but may be edited if required.

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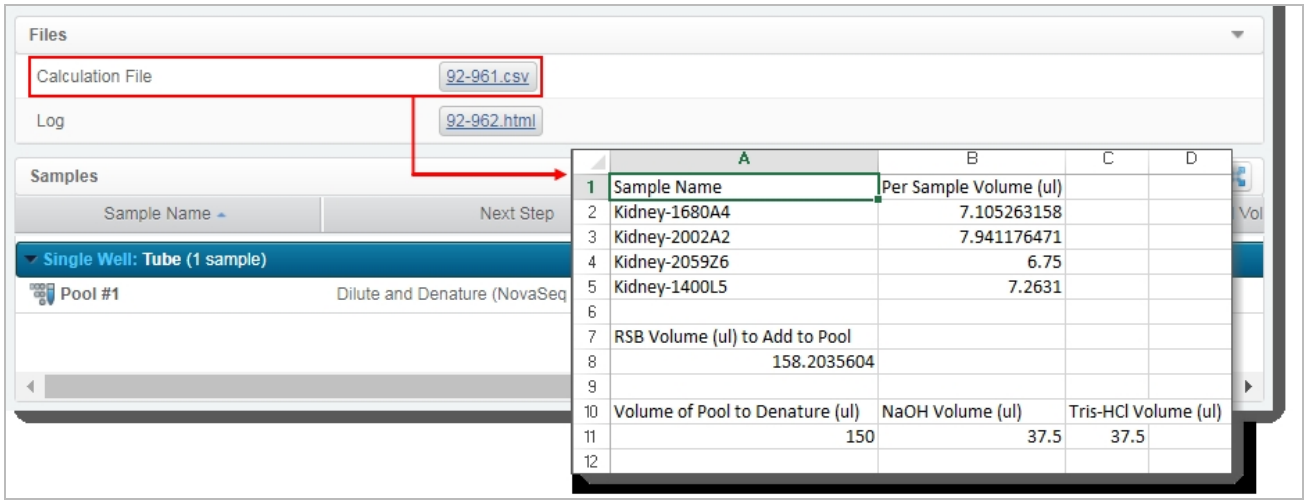


c. In the Sample Details table, the user can click on the pool icon to view details on the pool composition.



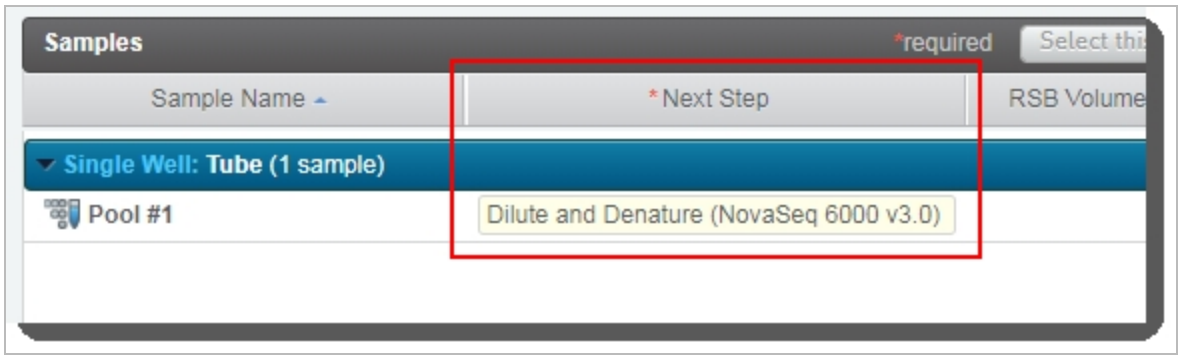
d. The user clicks Calculate Volumes. This triggers the Calculate Volumes automation, which performs volume calculations based on the selected Flowcell Type:
   - Calculates Bulk Pool Volume (ul) value.
   - Sets the value of the Bulk Pool Volume (ul) field, based on the selected Flowcell Type.
   - Calculates the Per Sample Volume (ul) value to be added to the pool.
   - Calculates the Total Sample Volume (ul) value.

- If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (ul) value.
- Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
- Populates the Volume of Pool to Denature (ul), NaOH Volume (ul) and T ris-HCl Volume (ul) columns of the Sample Details table. (Values are set by a script and are not editable by the user.)
- Generates the Calculation File (CSV) and attaches it to the step. This file contains volume information about the pool and the individual samples it contains.The user can click on the file to download it, and then open it in Excel.



- The user clicks Next Steps. This triggers the Set Next Step automation. This automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol - Dilute and Denature (NovaSeq 6000 v3.0).

e. On the Assign Next Steps screen: The next step is already set to Dilute and Denature (NovaSeq 6000 v3.0).



- User clicks Finish Step. At the end of this step, the pool of samples automatically advances to the Dilute and Denature (NovaSeq 6000 v3.0) step.

Step 2: Dilute and Denature (NovaSeq 6000 v3.0)

In this step, pooled samples are denatured and diluted by the addition of NaOH, Tris-HCl, and Resuspension Buffer (RSB), and are manually placed by the user into the library tube that will be used in the NovaSeq run.

In addition, this step validates the run setup information and generates the sample sheet file.

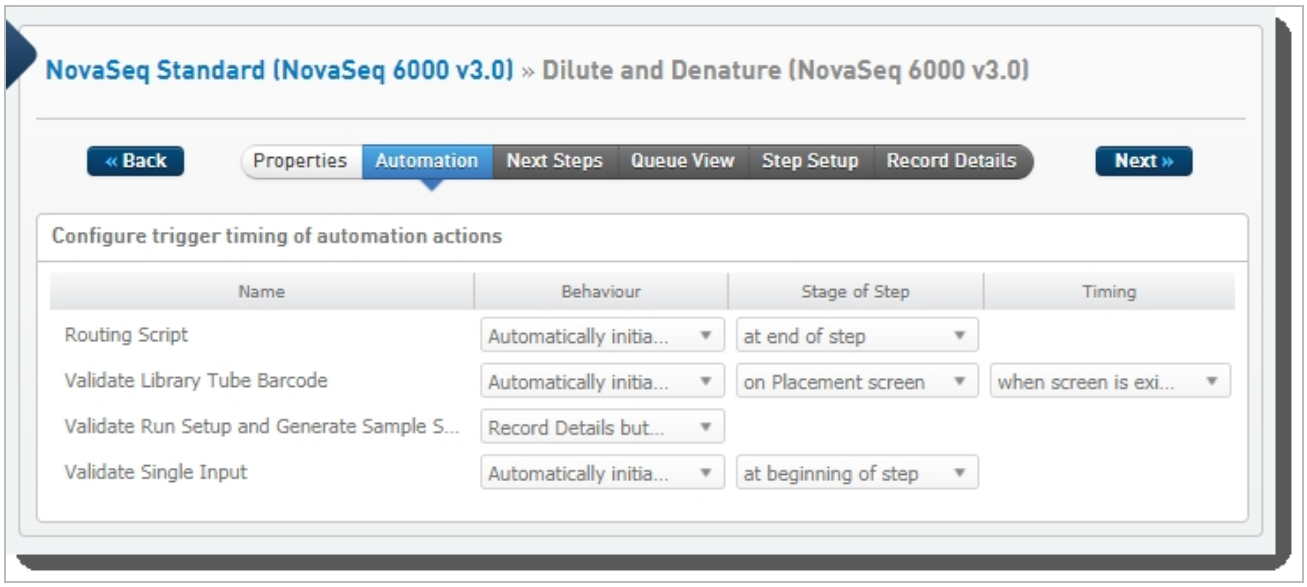
Step input: Bulk pool

Step output: Library tube

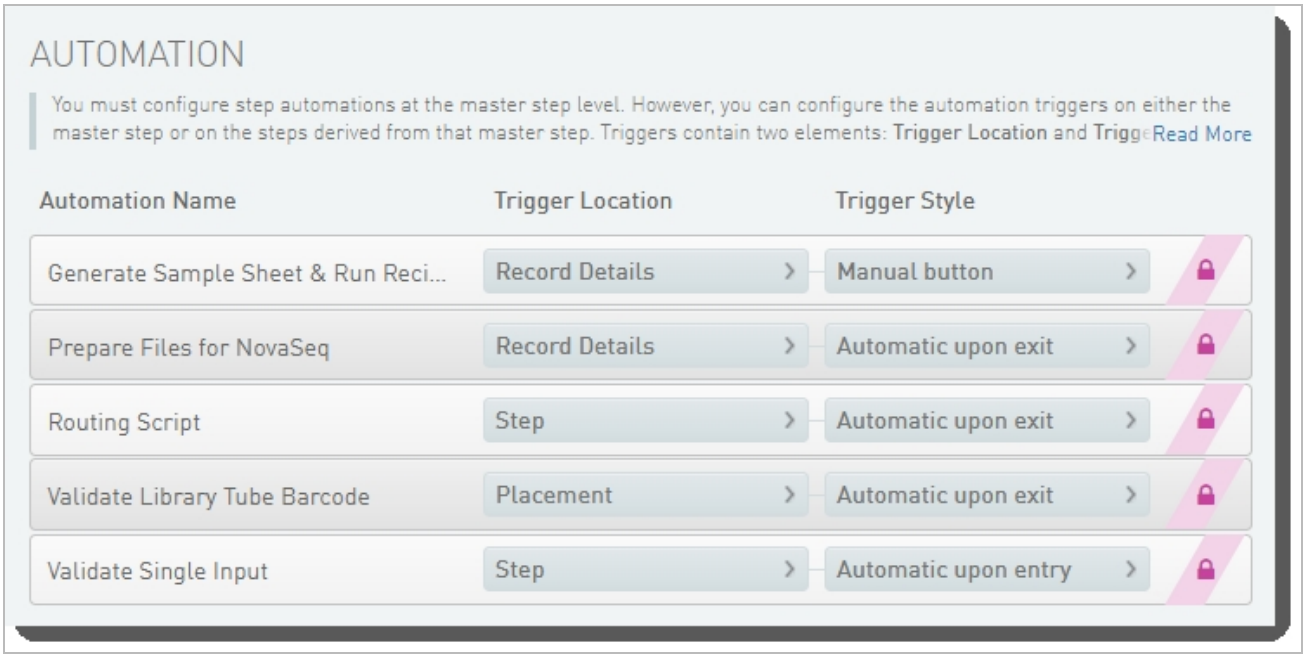
The following automations are configured on the step:

- Routing Script
- Validate Library Tube Barcode
- Validate Run Setup and Generate Sample Sheet
- Validate Single Input

Dilute and Denature (NovaSeq 6000 v3.0) automation configuration - LIMS v4.2.x:



Dilute and Denature (NovaSeq 6000 v3.0) automation configuration - LIMS v5.x:



These automations are described below, in the order in which they are triggered at run time.

Validate Single Input

Automatically triggered at the beginning of the step, this automation checks that there is only one container input to the step.

```
script:validateSampleCount -min 1 -max 1
```

Validate Library Tube Barcode

Automatically triggered on exit of the Placement screen, this automation:

- Validates the library tube barcode to ensure it conforms to the barcode mask [A-Za-z]{2}[0-9]{7}-[A-Za-z]{3}:

```
if (!output.container.name.matches(::[A-Za-z]{2}[0-9]{7}-[A-Za-z]{3}::)) { fail
(::Invalid Library Tube Barcode. Please verify and try again.::) }
```

- Copies Flowcell Type and Loading Workflow Type field values from step inputs to outputs:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ; output.::Loading Workflow
Type:: = input.::Loading Workflow Type::'
```

Validate Run Setup and Generate Sample Sheet

Automatically triggered when user clicks a button on the Record Details screen, this automation:

- Copies the Flowcell Type from the step input to the Run Mode field (hidden):

```
step.::Run Mode:: = input.::Flowcell Type::
```

- Validates the parameters entered on the Record Details screen, which will be used to set up the run and generate the sample sheet file (see *Respond to Validation/Recipe Request Call from Sequencer on page 863*, below).
  - Experiment Name may only contain alphanumeric, dash, or underscore characters. Spaces are not permitted.
  - When Workflow Type = No Index, Index Read 1 must be zero. For any other Workflow Type, Index Read 1 must be greater than zero.

```
if(!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) {
  fail(::Experiment Name contains prohibited characters. Allowed characters are:
a-z, A-Z, 0-9, -, and _::)
};
if(step.::Workflow Type::== ::No Index::) {
  if(step.::Index Read 1::!= 0) {
    fail(::Index Read 1 must be 0 if the Workflow Type is No Index.::)
  }
} else {
  if(step.::Index Read 1::== 0) {
    fail(::Index Read 1 must be greater than 0 if the Workflow Type is ::+
step.::Workflow Type::+ ::.::)
  }
};
```

- Checks the Paired End and Read 2 Cycles field values.
  - If Paired End = True and Read 2 Cycles value is 0, generates an error.

```
if (step.::Paired End::.toBoolean() && step.::Read 2 Cycles:: == 0) {
  fail(::Read 2 Cycles must not be zero if it is Paired End read.::)
};
```

- Checks the Workflow Type and Read 2 Cycles field values.
  - If Workflow Type is set to Dual Index and Index Read 2 value is 0, generates an error.
  - If Workflow Type is set to No Index or Single Index, sets Index Read 2 to 0.

```
if (step.::Workflow Type:: == ::Dual Index:: && step.::Index Read 2:: == 0) {
  fail(::Index Read 2 must not be zero if Workflow Type is Dual Index.::)
};
if (step.::Workflow Type:: == ::No Index:: || step.::Workflow Type:: == ::Single
Index::) {
  step.::Index Read 2:: = 0
};
```

- If Paired End = False, sets Read 2 Cycles value to 0.

```
if (!step.::Paired End::.toBoolean()) { step.::Read 2 Cycles:: = 0 };
```

- Checks the Flowcell Type field value.

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  - If Flowcell Type value is not SP, checks that the value of both Read 1 Cycles and Read 2 Cycles is 151 or less. If value is greater than 151, generates an error message.

```
if (input.::Flowcell Type::!=::SP:: && step.::Read 1 Cycles:: > 151) { fail
(::Read 1 Cycles must not be larger than 151 if it is not SPrime Flowcell::) };if
(input.::Flowcell Type::!=::SP:: && step.::Read 2 Cycles:: > 151) { fail(::Read 2
Cycles must not be larger than 151 if it is not SPrime Flowcell::)
```

- Sets the next step for samples to REMOVE:

```
nextStep = ::REMOVE::
```

- Generates the sample sheet and attaches it to the step (see Sample sheet generation section, below).

```
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p {password}
\   script:driver_file_generator \
  -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_BCL2FASTQ_
Samplesheet.csv \
  -o {compoundOutputFileLuid0}.csv \
  -q true \
  -destLIMSID {compoundOutputFileLuid0} \
  -l {compoundOutputFileLuid1}"
```

Routing Script

Automatically triggered on exit of the step, this automation invokes the changeWorkflow script, which routes step outputs to the NovaSeq 6000 v3.0 workflow, and queues them for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0) step.

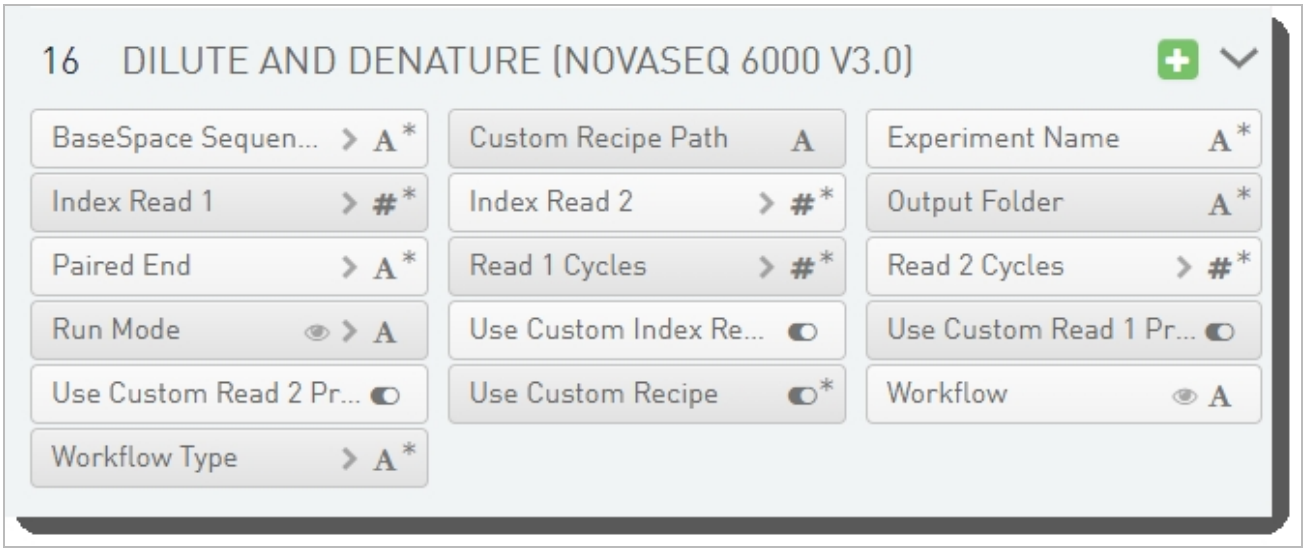
Default automation command line:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -u {username} -p {password} -i {stepURI:v2} -l
{compoundOutputFileLuid2} script:changeWorkflow \ \
  --FIELD_NAME 'N/A' \
  --FIELD_VALUE 'N/A' \
  --WORKFLOW 'NovaSeq 6000 v3.0' \
  --STEP 'AUTOMATED - NovaSeq Run (NovaSeq 6000 v30)' \
  --INPUTS_OR_OUTPUTS 'OUTPUTS'"
```

Step UDFs/Custom Fields

There are 16 fields defined on the Dilute and Denature (NovaSeq 6000 v3.0) step. These fields are required for sample sheet and JSON file generation.

The fields are shown below in the BaseSpace Clarity LIMS v5.x Configuration area (Custom Fields > Master Step Fields tab).



See the following table for LIMS v4.2.x and LIMS v5.x field configuration details:

Field Name	Field Type	Field Contraints/Options	Preset Values/Additional Options and Dropdown Items
BaseSpace Sequence Hub Configuration	Single-line Text (v4.2.x) Text Dropdown (v5.x)	• Required: Yes • Read Only: No • Custom Entries: No	• Not Used • Run Monitoring Only • Run Monitoring and Storage
Custom Recipe Path	Single-line Text (v4.2.x) Text (v5.x)	• Required: No • Read Only: No	
Experiment Name	Single-line Text (v4.2.x) Text (v5.x)	• Required: Yes • Read Only: No	
Index Read 1	Numeric (v4.2.x) Numeric Dropdown (v5.x)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 0 • 6 • 8 • Range From: 0 to 20 • Decimal Places Displayed: 0
Index Read 2	Numeric (v4.2.x) Numeric Dropdown (v5.x)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 0 • 6 • 8 • Range From: 0 to 20 • Decimal Places Displayed: 0

Field Name	Field Type	Field Contraints/Options	Preset Values/Additional Options and Dropdown Items
Output Folder	Single-line Text (v4.2.x) Text (v5.x)	• Required: Yes • Read Only: No	
Paired End	Single-line Text (v4.2.x) Text Dropdown (v5.x)	• Required: Yes • Read Only: No • Custom Entries: No	• True • False
Read 1 Cycles	Numeric (v4.2.x) Numeric Dropdown (v5.x)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 251* • 151 • 101 • 51 • Range From: 1 to 251 • Decimal Places Displayed: 0 **Value of 251 is only supported for SPflow cell type. For all other flow cell types, maximum value is 151.*
Read 2 Cycles	Numeric (v4.2.x) Numeric Dropdown (v5.x)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 251* • 151 • 101 • 51 • Range From: 1 to 251 • Decimal Places Displayed: 0 **Value of 251 is only supported for SPflow cell type. For all other flow cell types, maximum value is 151.*
Run Mode *(not displayed in user interface)*	Single-line Text (v4.2.x) Text Dropdown (v5.x)	• Required: No • Read Only: Yes • Custom Entries: No	• SP • S1 • S2 • S4

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Field Name	Field Type	Field Contraints/Options	Preset Values/Additional Options and Dropdown Items
Use Custom Index Read 1 Primer LIMS v5.0.5 and later: See *Configuration Update for NovaSeq Integration with Clarity LIMS v5.0.5 and Later* on page 1237	Check Box (v4.2.x) Toggle Switch (v5.x)	• Required: No • Read Only: No	• None Set
Use Custom Read 1 Primer LIMS v5.0.5 and later: See *Configuration Update for NovaSeq Integration with Clarity LIMS v5.0.5 and Later* on page 1237	Check Box (v4.2.x) Toggle Switch (v5.x)	• Required: No • Read Only: No	• None Set
Use Custom Read 2 Primer LIMS v5.0.5 and later: See *Configuration Update for NovaSeq Integration with Clarity LIMS v5.0.5 and Later* on page 1237	Check Box (v4.2.x) Toggle Switch (v5.x)	• Required: No • Read Only: No	• None Set
Use Custom Recipe	Check Box (v4.2.x) Toggle Switch (v5.x)	• Required: Yes • Read Only: No	• Default: No
Workflow	Single-line Text (v4.2.x) Text Dropdown (v5.x)	• Required: No • Read Only: Yes	• GenerateFASTQ
Workflow Type	Single-line Text (v4.2.x) Text Dropdown (v5.x)	• Required: Yes • Read Only: No • Custom Entries: Yes	• No Index • Single Index • Dual Index • Custom

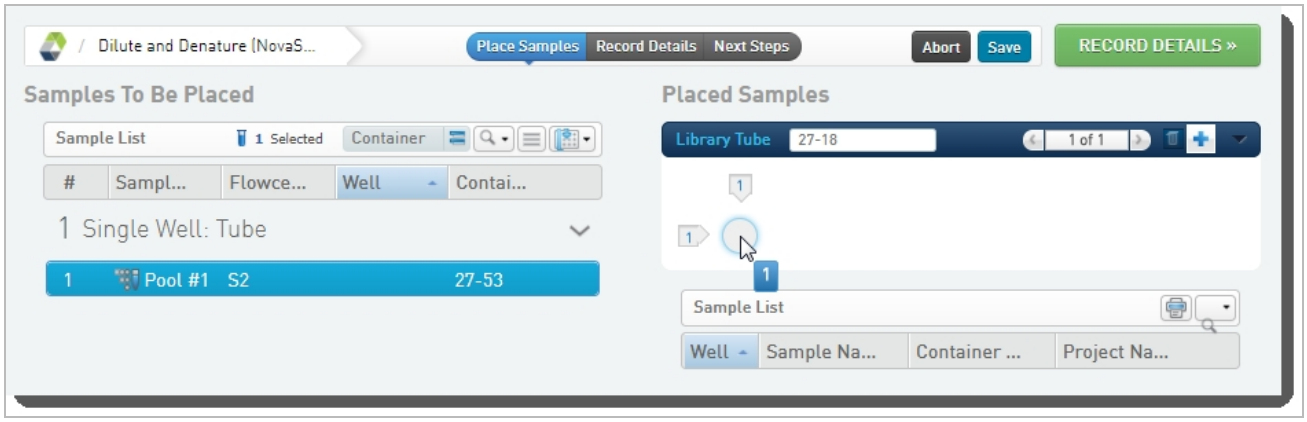
Global UDFs/Custom Fields

The following table lists the global UDFs/custom fields that are configured to display on the Dilute and Denature (NovaSeq 6000 v3.0) step.

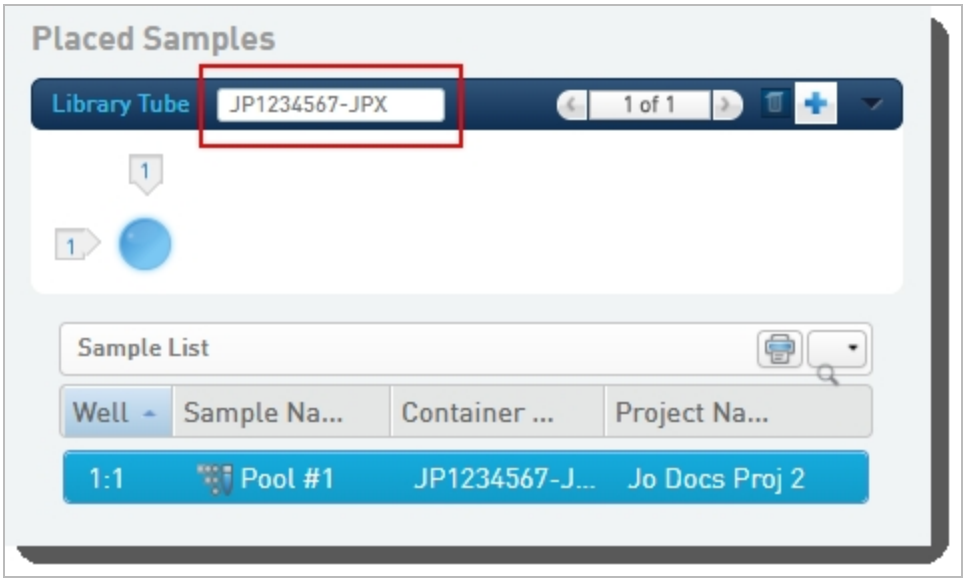
Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Configured on Analyte in LIMS v4.2.x and Derived Samplein LIMS v5.x:			
Flowcell Type	Single-line Text (v4.2.x) Text Dropdown (v5.x)	• Required: Yes • Read Only: No • Custom Entries: No	• SP • S1 • S2 • S4
Loading Workflow Type	Single-line Text (v4.2.x) Text Dropdown (v5.x)	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User Interaction

1. On the Placement screen, user does the following:
    - Drags the pool into the library tube in the Placed Samples area.



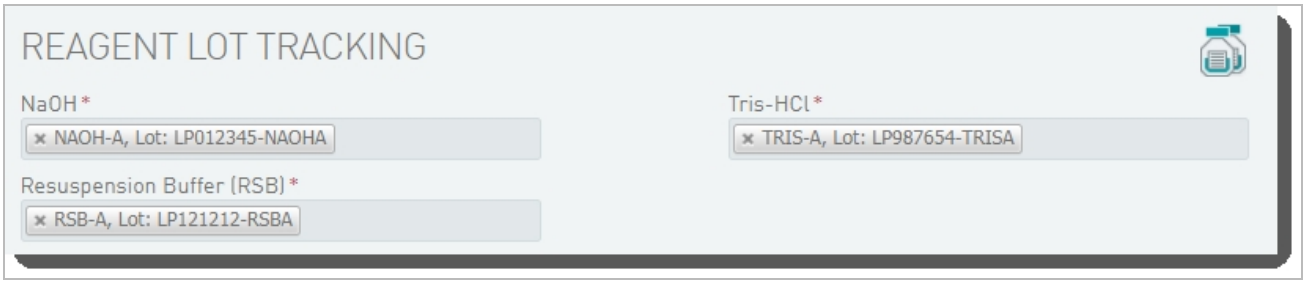
    - Scans or types the barcode of the library tube into the Library Tube field.



- Clicks Record Details.

On exit of the Placement screen, the Validate Library Tube Barcode automation checks that the library tube barcode conforms to the barcode mask [A-Za-z]{2}[0-9]{7}-[A-Za-z]{3}, and displays an error message if this is not the case.

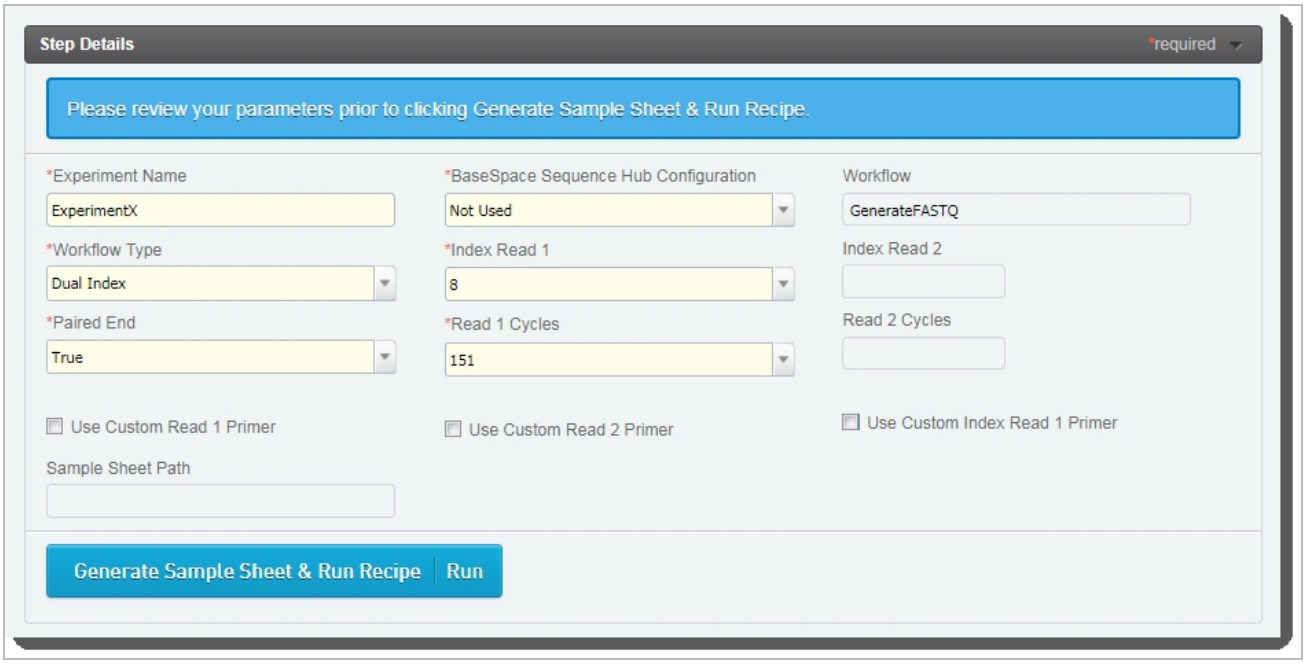
2. On the Record Details screen:
   - The Reagent Lot Tracking section lets you track the NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. The user selects from the active lots displayed in each drop-down list.



To make reagent lots available for selection in the Reagent Lot Tracking section, you must first configure and activate them. You can do this on the Reagents and Controls screen (LIMS v4.2.x) or the Configuration > Consumables screen (LIMS v5.x).

- The fields displayed on the Record Details screen are used to set up the run and generate the sample sheet file.

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Some of these fields are auto-populated and some must be completed by the user (see the following table for details):

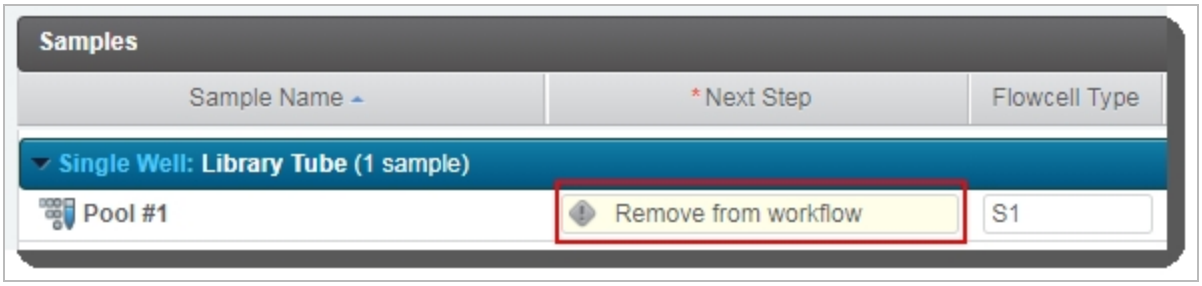
Field	Value
Experiment Name	User enters the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.
BaseSpace Sequence Hub Configuration	User selects from preset options: Not Used, Run Monitoring Only, Run Monitoring and Storage
Workflow	Automatically populated with preset - GenerateFASTQ
Workflow Type	User selects from preset options: No Index, Single Index, Dual Index, Custom
Index Read 1	User selects from preset options: 0, 6, 8 - or type a value between 0 and 20
Index Read 2	User selects from preset options: 0, 6, 8 - or type a value between 0 and 20
Paired End	User selects from preset options: True or False
Read 1 Cycles	User selects from preset options: 251, 151, 101, 51 - or type a value between 1 and 251 Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Read 2 Cycles	User selects from preset options: 251, 151, 101, 51 - or type a value between 1 and 251 Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.

Field	Value
Use Custom Read 1 Primer	User selects if applicable.
Use Custom Read 2 Primer	User selects if applicable.
Use Custom Index Read 1 Primer	User selects if applicable.
Output Folder	User enters the network path for sequencing run folder. For example: \\networkshare\run_data
Use Custom Recipe	User selects if applicable.
Custom Recipe Path	If Use Custom Recipe selected, user enters the path to the custom recipe file to be used.

3. On the Record Details screen, user clicks Validate Run Setup and Generate Sample Sheet. This triggers the automation script, which does the following:
    - Validates the information provided to set up the run.
    - Generates the sample sheet file and attaches it to the placeholder in the Files area of the Record Detailsscreen.



    - Sets the value of the next step to Remove from workflow. The Routing Script automation expects this value, and requires it in order to successfully advance samples to the next step.
    - User clicks Next Steps.
4. On the Assign Next Steps screen: The Next Step field for all samples is pre-populated with Remove from workflow.



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The user must not change this value. If Next Step is not set to Remove from workflow, the routing script will not be able to route samples correctly.

- User clicks Finish Step.

5. On exit from the step:
    - The Routing Script automation is triggered and samples are routed to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0) protocol.
    - In Lab View, the pool of samples is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0) step.

At this point in the workflow, the user interaction ends. The integration automatically starts the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0) step and data from the run is parsed back into the LIMS.

Protocol 3: NovaSeq Xp (NovaSeq 6000 v3.0)

Samples are routed to this protocol if their Loading Workflow Type value is set to NovaSeq Xp.

Samples are pooled and added to lanes on the NovaSeq flow cell type selected in the Define Run Format (NovaSeq 6000 v3.0) step. At the end of this protocol, the flow cell is sent to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0) protocol.

This protocol contains three steps, described below:

- Step 1: Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.0)
- Step 2: Dilute, Denature and ExAmp (NovaSeq 6000 v3.0)
- Step 3: Load to Flowcell (NovaSeq 6000 v3.0)

Step 1: Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.0)

In this step, user manually places libraries into a pool.

User must only create one pool per step.

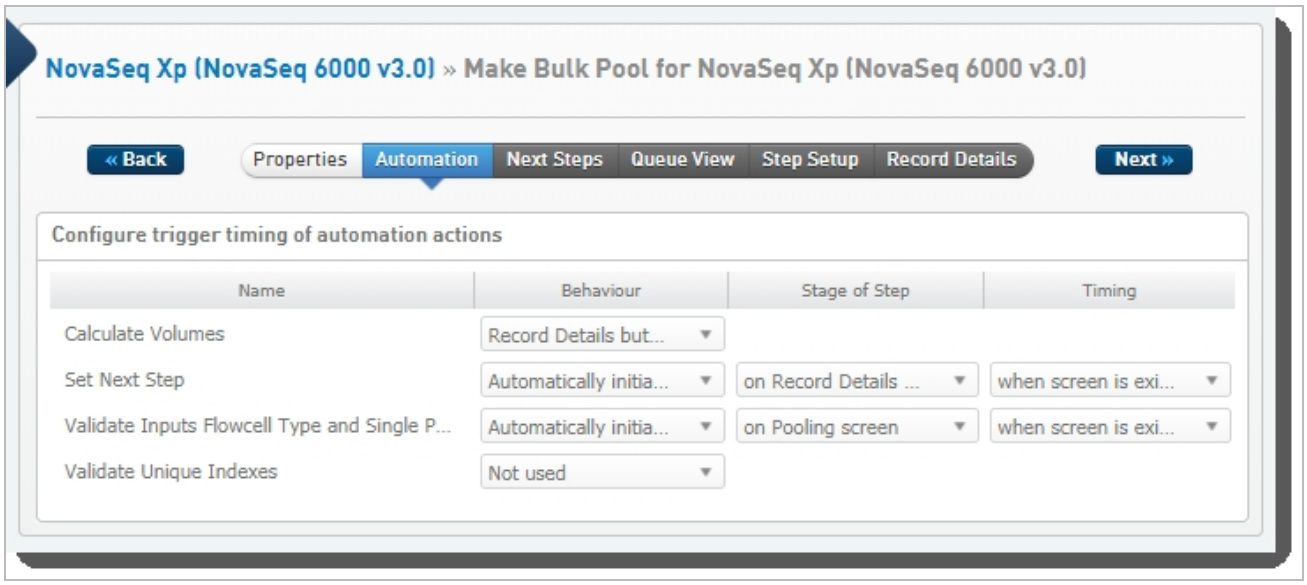
Step input: NTP (normalized libraries)

Step output: Bulk pool

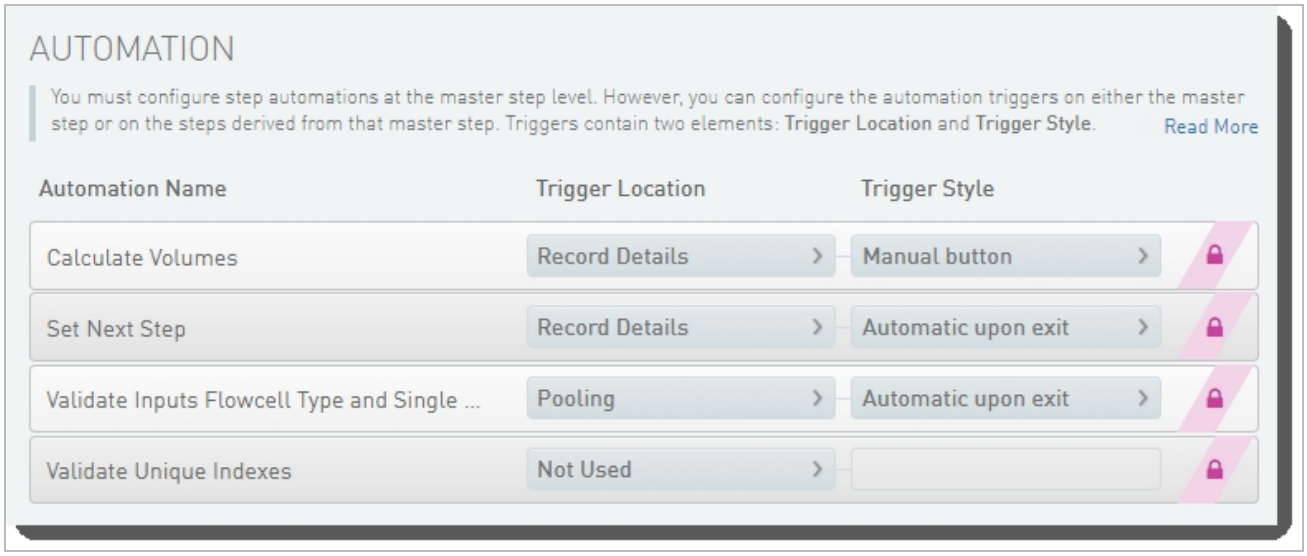
The following automations are configured on the step:

- Calculate Volumes
- Set Next Step
- Validate Inputs Flowcell Type and Single Pool
- Validate Unique Indexes *(Not used. This functionality is handled by the core LIMS configuration for pooling.)*

Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.0) automation configuration - LIMS v4.2.x:



Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.0) automation configuration - LIMS v5.x:



These automations are described below, in the order in which they are triggered at run time.

Validate Inputs Flowcell Type and Single Pool

Automatically triggered on exit of the Pooling screen, this automation:

- Checks that all samples in the pool have the same Flowcell Type assigned to them.
- Checks that only one pool has been created.
- Changes the value of error and logging messages to reference the type of pool passed as 'bulk' pool.

```
/opt/gls/clarity/bin/java \-jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar \script:validate_flowcell_for_input_pools \
```

```
  -i {stepURI:v2} \
  -u {username} \
  -p {password} \
  -l {compoundOutputFileLuid1} \
  -validateSingleOutput true \
  -poolType bulk"
```

Calculate Volumes

Automatically triggered when the user clicks the Calculate Volumes button on the Record Details screen, this automation:

- Calculates the number of samples in the pool:

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: + 1
```

- Sets the value of the Bulk Pool Volume (ul) field,based on the selected Flowcell Type:

```
if ( input.::Flowcell Type:: == ::SP:: ) {
  step.::Bulk Pool Volume (ul):: = step.::Number of Lanes to Sequence:: * 18 + 30
} ;
if ( input.::Flowcell Type:: == ::S1:: ) {
  step.::Bulk Pool Volume (ul):: = step.::Number of Lanes to Sequence:: * 18 + 30
} ;
if ( input.::Flowcell Type:: == ::S2:: ) {
  step.::Bulk Pool Volume (ul):: = step.::Number of Lanes to Sequence:: * 22 + 30
} ;
if ( input.::Flowcell Type:: == ::S4:: ) {
  step.::Bulk Pool Volume (ul):: = step.::Number of Lanes to Sequence:: * 30 + 30
} ;
```

- Calculates the Per Sample Volume (ul) to be added to the pool:

```
input.::Per Sample Volume (ul)::= (((input.::Final Loading Concentration (pM)::*
5/ 1000) / input.::Normalized Molarity (nM)::) * step.::Bulk Pool Volume (ul)::)
/ step.::Number of Samples in Pool::
```



To ensure accurate pipetting of each sample in a pool for sequencing, the Per Sample Volume (ul) value must be equal to or higher than the Minimum Per Sample Volume (ul) (default value set at5, may be edited by the user).

Assuming the default Minimum Per Sample Volume (ul) value of 5, for a given batch:

1. If the smallest Per Sample Volume (ul) value is less than 5, the LIMS automatically assigns a value of 5 to the sample's Adjusted Per Sample Volume (ul) field.
2. The LIMS then adjusts the Adjusted Per Sample Volume (ul) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

- Calculates the Total Sample Volume (ul) field value:

```
step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: +
input.::Adjusted Per Sample Volume (ul)::
```

- If the Total Sample Volume is less than the Bulk Pool Volum e, calculates the RSB Volume (ul) field value:

```
if (step.::Total Sample Volume (ul):: >= step.::Bulk Pool Volume (ul)::)
{output.::RSB Volume (ul):: = 0} else {output.::RSB Volume (ul):: = step.::Bulk
Pool Volume (ul):: - step.::Total Sample Volume (ul)::} ;
```

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs:

```
output.::Flowcell Type::= input.::Flowcell Type::;output.::Loading Workflow
Type::= input.::Loading Workflow Type::;
```

- Uses the NovaSeq_Xp_Bulk_Pool.csv and NovaSeq_Xp_Bulk_Pool2.csv template files to generate a single CSV file containing information about the bulk pool and the samples it contains. The generated file is available for download by the user on the Step Setup screen of the following step - Dilute, Denature and ExAmp (NovaSeq 6000 v3.0).

```
&& /opt/gls/clarity/bin/java \-jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar \
  -i {stepURI:v2} \
  -u {username} \
  -p {password} \
  -l {compoundOutputFileLuid1} \ script:driver_file_generator \
  -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_Bulk_
Pool.csv \
  -o {compoundOutputFileLuid0}.csv \ script:driver_file_generator \
  -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_Bulk_
Pool2.csv \
  -o append.csv \
&& cat append.csv >> {compoundOutputFileLuid0}.csv"
```

Set Next Step

Automatically triggered on exit of the Record Details screen, this automation:

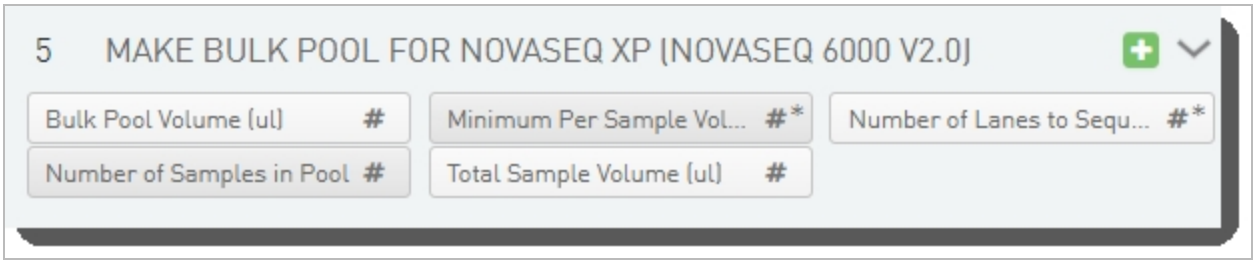
- Copies the Flowcell Type values from the step inputs to the step outputs.
- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ; nextStep = ::ADVANCE::
```

Step UDFs/Custom Fields

There are five fields defined on the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.0) step.

These are shown below in the BaseSpace Clarity LIMS v5.x Configuration area (Custom Fields > Master Step Fields tab).



See the following table for LIMS v4.2.x and LIMS v5.x field configuration details:

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Bulk Pool Volume (ul) (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2
Number of Lanes to Sequence	Numeric	• Required: Yes • Read Only: No	• Decimal Places Displayed: 0
Total Sample Volume (ul) (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Default: 0 • Decimal Places Displayed: 0
Minimum Per Sample Volume (uL)	Numeric	• Required: Yes • Read Only: No	• Default: 5 • Decimal Places Displayed: 2
Number of Samples in Pool (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Default: 0 • Decimal Places Displayed: 0

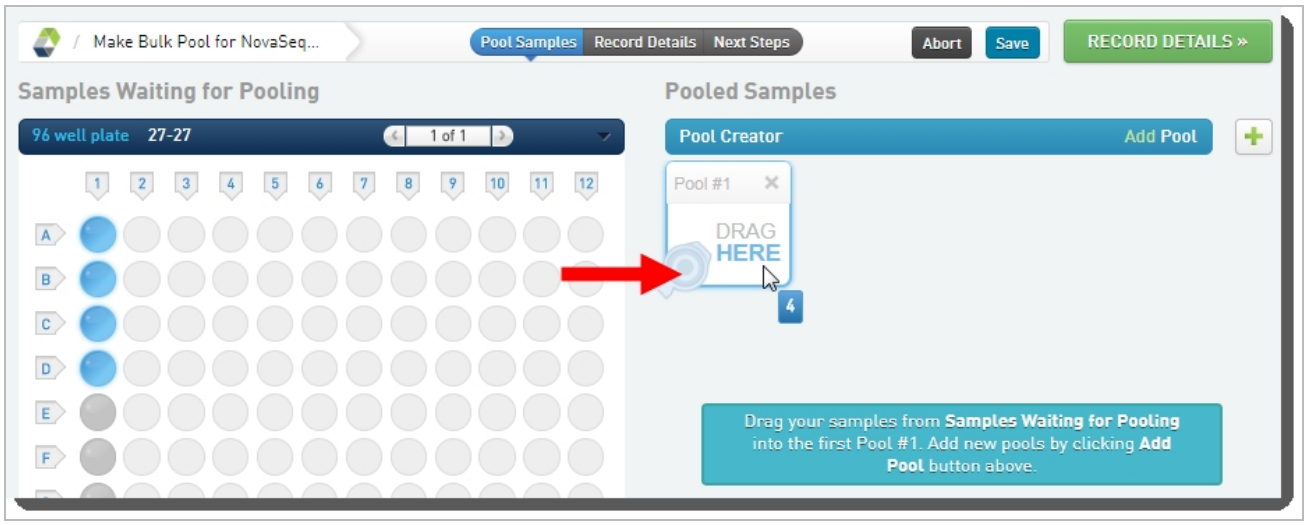
Global UDFs/Custom Fields

The following table lists the global UDFs/custom fields that are configured to display on the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.0) step:

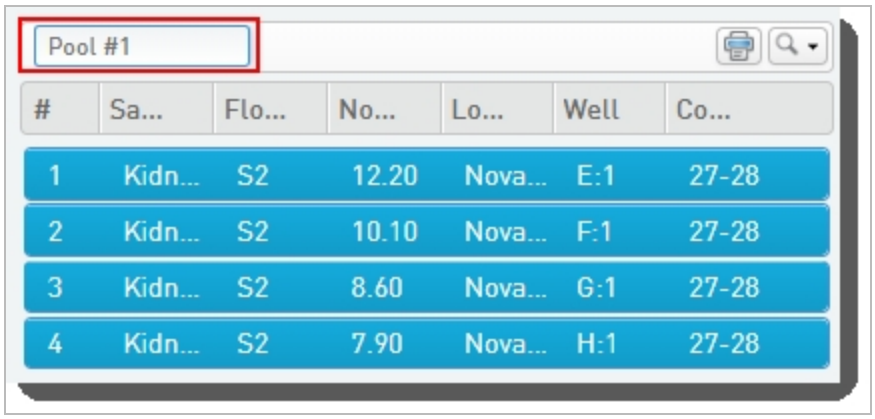
Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Configured on Analyte in LIMS v4.2.x and Derived Samplein LIMS v5.x:			
RSB Volume (ul)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Flowcell Type	Single-line Text (v4.2.x) Text Dropdown (v5.x)	• Required: Yes • Read Only: No • Custom Entries: No	• SP • S1 • S2 • S4
Loading Workflow Type	Single-line Text (v4.2.x) Text Dropdown (v5.x)	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User Interaction

1. On the Queue screen, user adds samples of the same Flowcell Type to the Ice Bucket and clicks View Ice Bucket.
2. On the Ice Bucket screen, user clicks Begin Work.
3. On the Pooling screen, user:
    - Drags samples over into the Pool Creator to create a pool.



    - Names the pool, or accepts the default name - Pool #1.

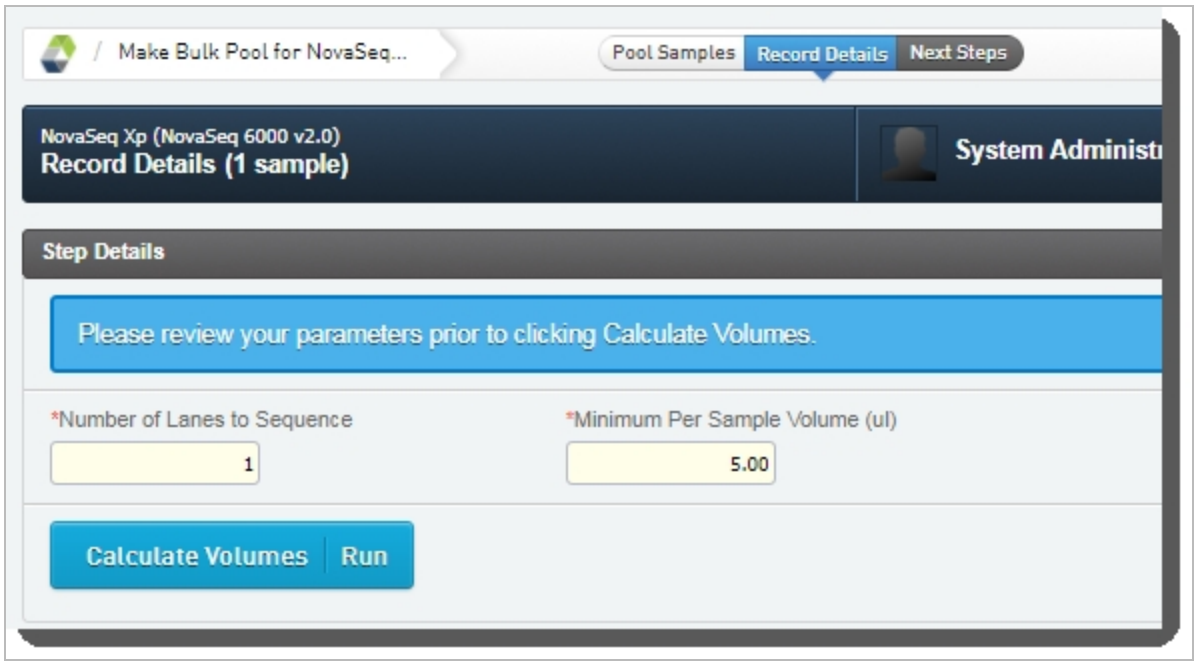


- Clicks Record Details.

On exit from the Pooling screen, the Validate Inputs Flowcell Type and Single Pool is triggered. The automation checks that all samples in the pool have been assigned the same Flowcell Type, and only one pool has been created.

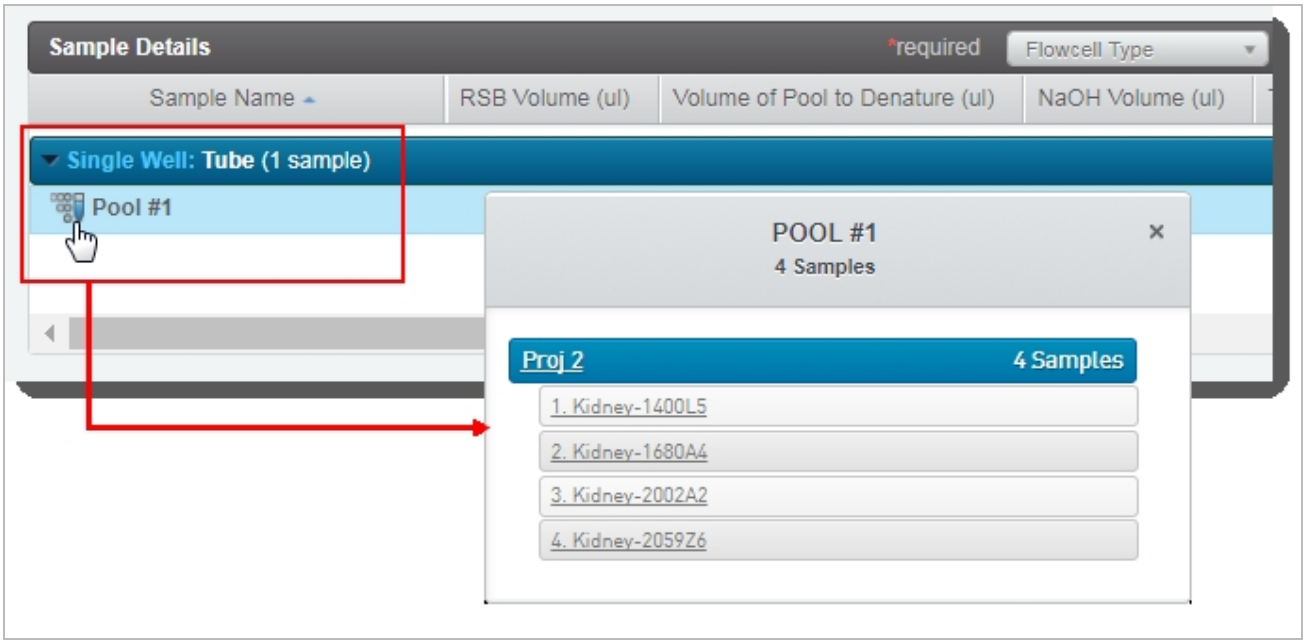
4. On the Record Details screen, the Step Details area contains two required fields:
   - Number of Lanes to Sequence: The value entered in this field is used in volume calculations, to ensure that volumes are sufficient for the number of times the pool will be sequenced.
   - Minimum Per Sample Volume (ul): In many applications, and particularly where robotics are used, a minimum sample volume is required. The value in this field is used to calculate how much of each sample will be included in the pool.

   The field is pre-populated with the configured default value, 5 ul, but may be edited if required.

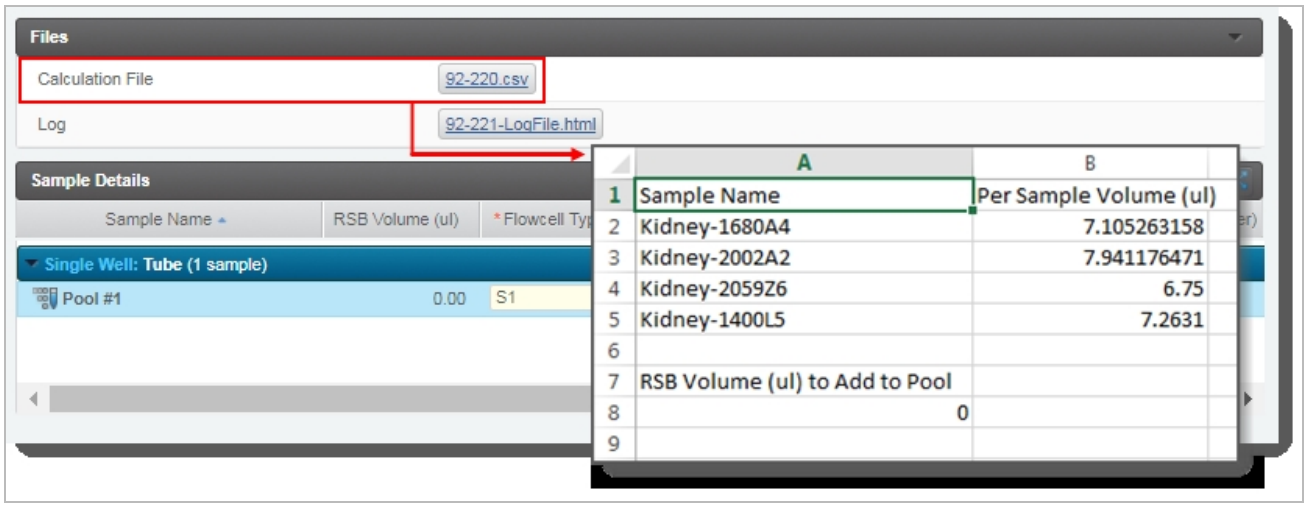


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- In the Sample Details table, the user can click on the pool icon to view details on the pool composition.



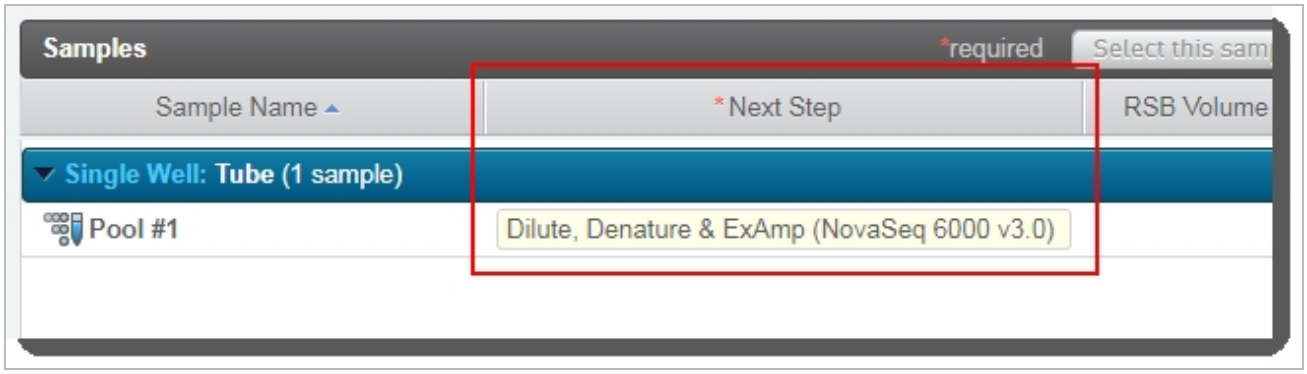
- The user clicks Calculate Volumes. This triggers the Calculate Volumes automation, which performs volume calculations based on the selected Flowcell Type: Calculates Bulk Pool Volume (ul) value.
  - Calculates the Per Sample Volume (ul) value to be added to the pool.
  - Calculates the Total Sample Volume (ul) value.
  - If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (ul) value.
  - Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
  - Generates the Calculation File (CSV) and attaches it to the step. This file contains volume information about the samples and RSB buffer to add to the pool.The user can click on the file to download it, and then open it in Excel.



– The user clicks Next Steps. This triggers the Set Next Step automation. This automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol - Dilute, Denature and ExAmp (NovaSeq 6000 v3.0).

5. On the Assign Next Steps screen:

- The next step is already set to Dilute, Denature and ExAmp (NovaSeq 6000 v3.0).



- User clicks Finish Step. At the end of this step, the pool of samples automatically advances to the Dilute, Denature and ExAmp (NovaSeq 6000 v3.0) step.

Step 2: Dilute, Denature and ExAmp (NovaSeq 6000 v3.0)

In this step, pooled samples are denatured and diluted by the addition of DPX, NaOH, Tris-HCl, and RSB. The user manually creates working pools based on the number of lanes they want to sequence.

Step input: Bulk pool

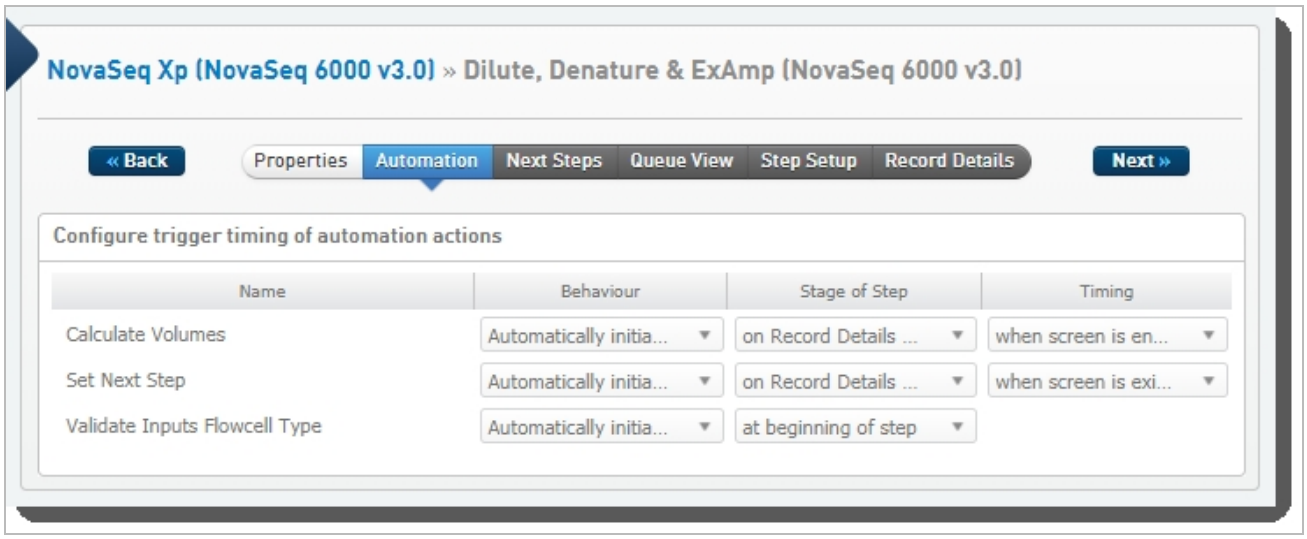
Step output: Working pool - variable number, the user chooses how many working pools to create per bulk pool

The following automations are configured on the step:

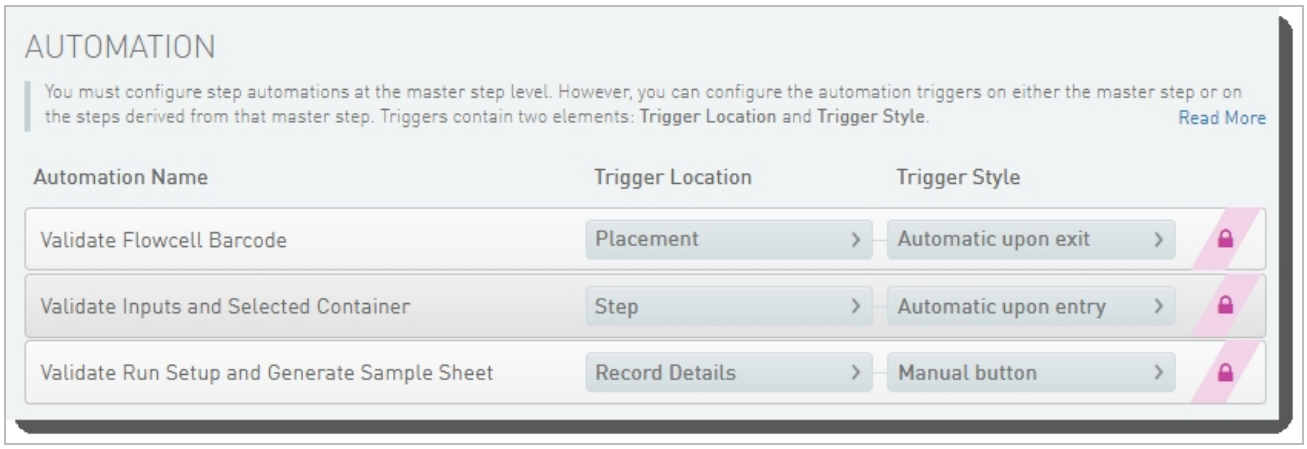
- Calculate Volumes
- Set Next Step

- Validate Inputs Flowcell Type

Dilute, Denature and ExAmp (NovaSeq 6000 v3.0) automation configuration - LIMS v4.2.x:



Dilute, Denature and ExAmp (NovaSeq 6000 v3.0) automation configuration - LIMS v5.x:



These automations are described below, in the order in which they are triggered at run time.

Validate Inputs Flowcell Type

Automatically triggered at the beginning of the step, this automation invokes the validate_flowcell_for_input_pools script. This script does the following:

- Checks the inputs to the step and validates that the Flowcell Type field has been set to a valid value (SP, S1, S2, or S4), and that all inputs have the same value for this field.
- The container type selected matches the value in the Flowcell Type field.
- Validates that the number of outputs matches the number of lanes on the selected flow cell type. If validation fails, an error message informs the user that the number of working pools does not match the number of lanes available on the flow cell.

```
bash -l -c "/opt/gls/clarity/bin/java \
-jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar
\script:validate_flowcell_for_input_pools \
  -i {stepURI:v2} \
  -u {username} \
  -p {password} \
  -l {compoundOutputFileLuid1}"
```

Calculate Volumes

Automatically triggered on entry to of the Record Details screen, this automation sets the value of the following fields, based on the Flowcell Type:

- BP Aliquot Volume (ul)
- NaOH Volume (ul)
- Tris-HCl Volume (ul)
- DPX1 Volume (ul)
- DPX2 Volume (ul)
- DPX3 Volume (ul)
- Mastermix per Lane (ul)

```
'if ( input.::Flowcell Type:: == ::SP:: ) {
  output.::BP Aliquot Volume (ul):: = 18 ;
  output.::NaOH Volume (ul):: = 4.50 ;
  output.::Tris-HCl Volume (ul):: = 4.50 ;
  step.::DPX1 Volume (ul):: = 126 ;
  step.::DPX2 Volume (ul):: = 18 ;
  step.::DPX3 Volume (ul):: = 66 ;
  output.::Mastermix per Lane (ul):: = 63};
if (input.::Flowcell Type:: == ::S1::) {
  output.::BP Aliquot Volume (ul):: = 18;
  output.::NaOH Volume (ul):: = 4.50;
  output.::Tris-HCl Volume (ul):: = 4.50;
  step.::DPX1 Volume (ul):: = 126;
  step.::DPX2 Volume (ul):: = 18;
  step.::DPX3 Volume (ul):: = 66;
  output.::Mastermix per Lane (ul):: = 63};
if (input.::Flowcell Type:: == ::S2::) {
  output.::BP Aliquot Volume (ul):: = 22;
  output.::NaOH Volume (ul):: = 5.50;
  output.::Tris-HCl Volume (ul):: = 5.50;
```


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```
  step.::DPX1 Volume (ul):: = 126;
  step.::DPX2 Volume (ul):: = 18;
  step.::DPX3 Volume (ul):: = 66;
  output.::Mastermix per Lane (ul):: = 77};
if (input.::Flowcell Type:: == ::S4::) {
  output.::BP Aliquot Volume (ul):: = 30;
  output.::NaOH Volume (ul):: = 7.50;
  output.::Tris-HCl Volume (ul):: = 7.50;
  step.::DPX1 Volume (ul):: = 315;
  step.::DPX2 Volume (ul):: = 45;
  step.::DPX3 Volume (ul):: = 165;
  output.::Mastermix per Lane (ul):: = 105
};
```

The automation also:

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs:

```
output.::Flowcell Type::= input.::Flowcell Type::;
output.::Loading Workflow Type::= input.::Loading Workflow Type::;
```

- Uses the NovaSeq_Xp_Working_Pool.csv and NovaSeq_Xp_Working_Pool2.csv template files to generate a single CSV file containing information about the DPX volume, and the volume of BP Aliquot, Mastermix, NaOH, and Tris-HCl to add per working pool. The generated file is available for download by the user on the Step Setup screen of the following step - Load to Flowcell (NovaSeq 6000 v3.0).

```
and /opt/gls/clarity/bin/java \
-jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/DriverFileGenerator.jar \
 -i {stepURI:v2} \
 -u {username} \
 -p {password} \
 -l {compoundOutputFileLuid1} \script:driver_file_generator \
 -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_Working_
Pool.csv \
 -o {compoundOutputFileLuid0}.csv \script:driver_file_generator \
 -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_Xp_Working_
Pool2.csv \
 -o append.csv \
&& cat append.csv >> {compoundOutputFileLuid0}.csv"
```

Set Next Step

Automatically triggered on exit of the Record Details screen, this automation:

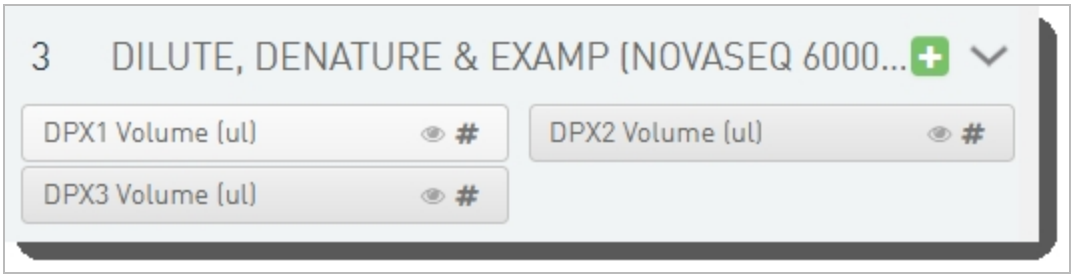
- Copies the Flowcell Type values from the step inputs to the step outputs.
- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ; nextStep = ::ADVANCE::
```

Step UDFs/custom fields

There are 3 fields defined on the Dilute, Denature and ExAmp (NovaSeq 6000 v3.0) step. (These field values are set by a script and are not editable by the user running the step.)

The fields are shown below in the BaseSpace Clarity LIMS v5.x Configuration area (Custom Fields > Master Step Fields tab).



See the following table for LIMS v4.2.x and LIMS v5.x field configuration details:

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
DPX1 Volume (ul)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0
DPX2 Volume (ul)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0
DPX3 Volume (ul)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0

Global UDFs/Custom Fields

The following table lists the global UDFs/custom fields that are configured to display on the Dilute, Denature and ExAmp (NovaSeq 6000 v3.0) step:

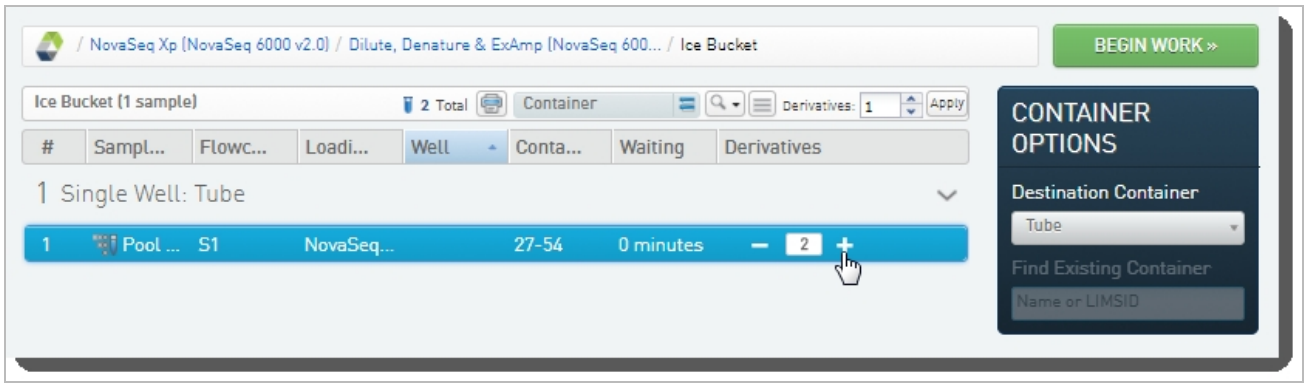
Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Configured on Analyte in LIMS v4.2.x and Derived Sample in LIMS v5.x:			

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
BP Aliquot Volume (ul)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0
Flowcell Type	Single-line Text (v4.2.x) Text Dropdown (v5.x)	• Required: Yes • Read Only: No • Custom Entries: No	• SP • S1 • S2 • S4
Loading Workflow Type	Single-line Text (v4.2.x) Text Dropdown (v5.x)	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
Mastermix per Lane (ul)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0
NaOH Volume (ul)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Tris-HCl Volume (ul)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2

User Interaction

1. On the Queue screen, user adds the pool to the Ice Bucket and clicks View Ice Bucket.
2. On the Ice Bucket screen, user (optionally) sets the number of derivatives to create - these will be placed into the flow cell lanes, and clicks Begin Work.



On entry to the Record Details screen, the Calculate Volumes automation is triggered. This automation sets the following values based on the selected Flowcell Type (calculation details are provided inCalculate Volumes automation section, above):

- BP Aliquot Volume (ul)

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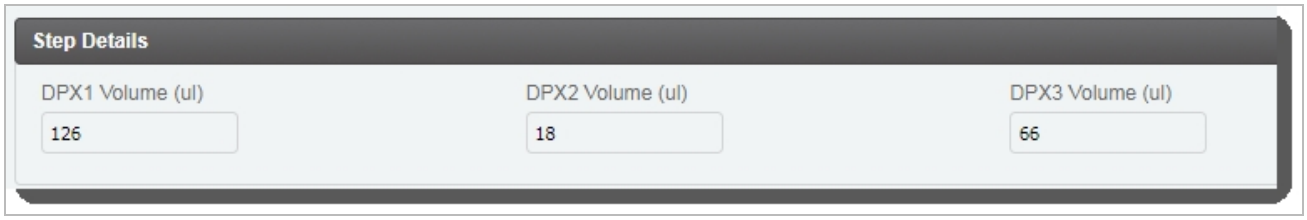
- NaOH Volume (ul)
- Tris-HCl Volume (ul)
- DPX1 Volume (ul)
- DPX2 Volume (ul)
- DPX3 Volume (ul)
- Mastermix per Lane (ul)
- Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
- Generates the Calculation File (CSV) and attaches it to the step. This file contains information about the DPX Mastermix volume, and the volume of Mastermix, NaOH, and Tris-HCl to add per working pool. The user can click on the file to download it and then open it in Excel.

3. On the Record Details screen: The Reagent Lot Tracking section lets you track the DPX1, DPX2, DPX3, NaOH, RSB, and Tris-HCl reagent lots. The user selects from the active lots displayed in each drop-down list.

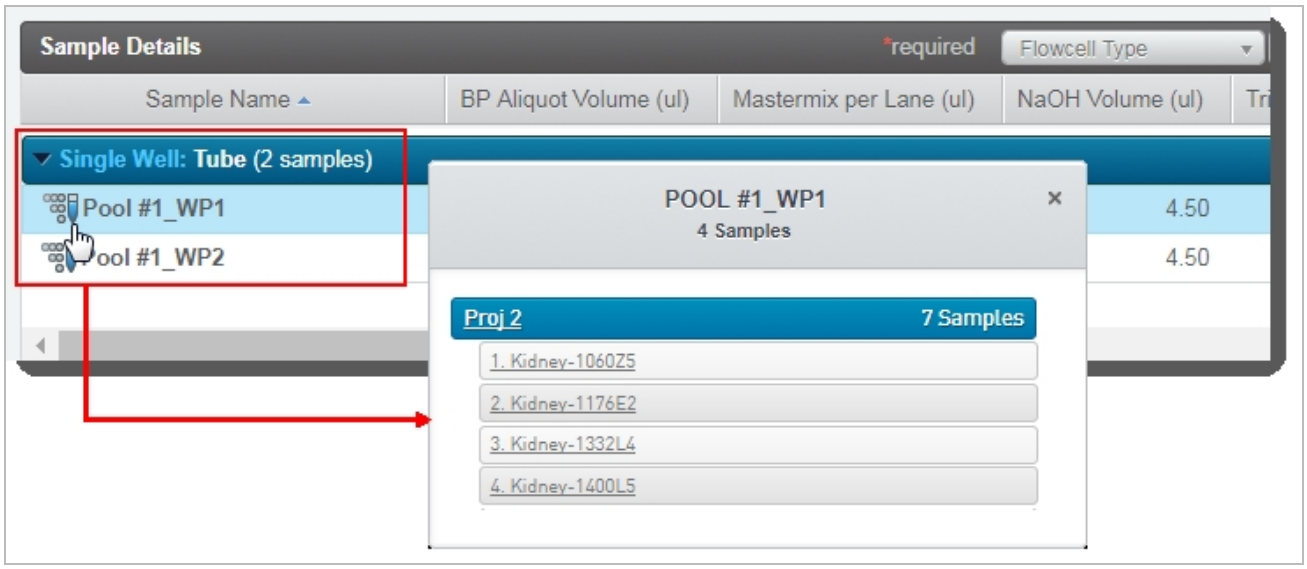


To make reagent lots available for selection in the Reagent Lot Tracking section, you must first configure and activate them. You can do this on the Reagents and Controls screen (LIMS v4.2.x) or the Configuration > Consumables screen (LIMS v5.x).

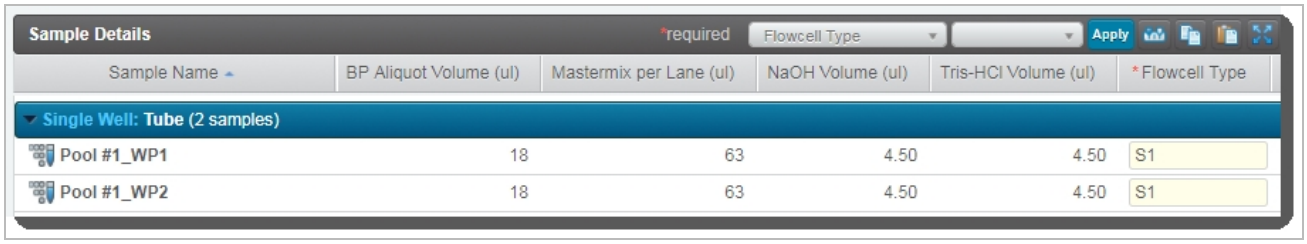
- In the Step Details area, the DPX1, DPX2, and DPX3 reagent volume values are already populated. (These values are set by a script and are not editable by the user running the step.)



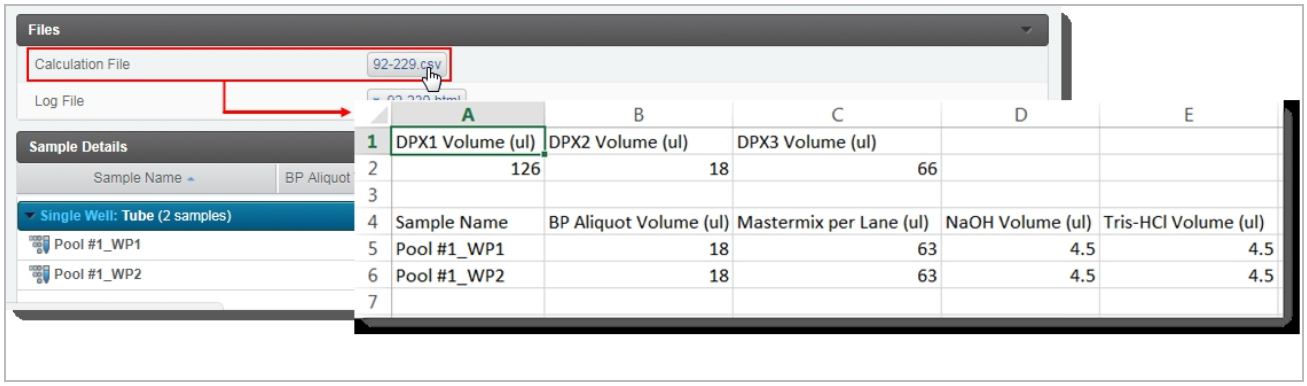
- In the Sample Details table:
  - The user can click on the pool icon to view details on the working pool composition.



- The BP Aliquot, Mastermix per lane, NaOH, and Tris-HCI volume values for each working pool are already populated. (These values are set by a script and are not editable by the user running the step.)
- The working pool number is appended to the bulk pool name. This allows users to quickly identify which working pools are derived from the same bulk pool.

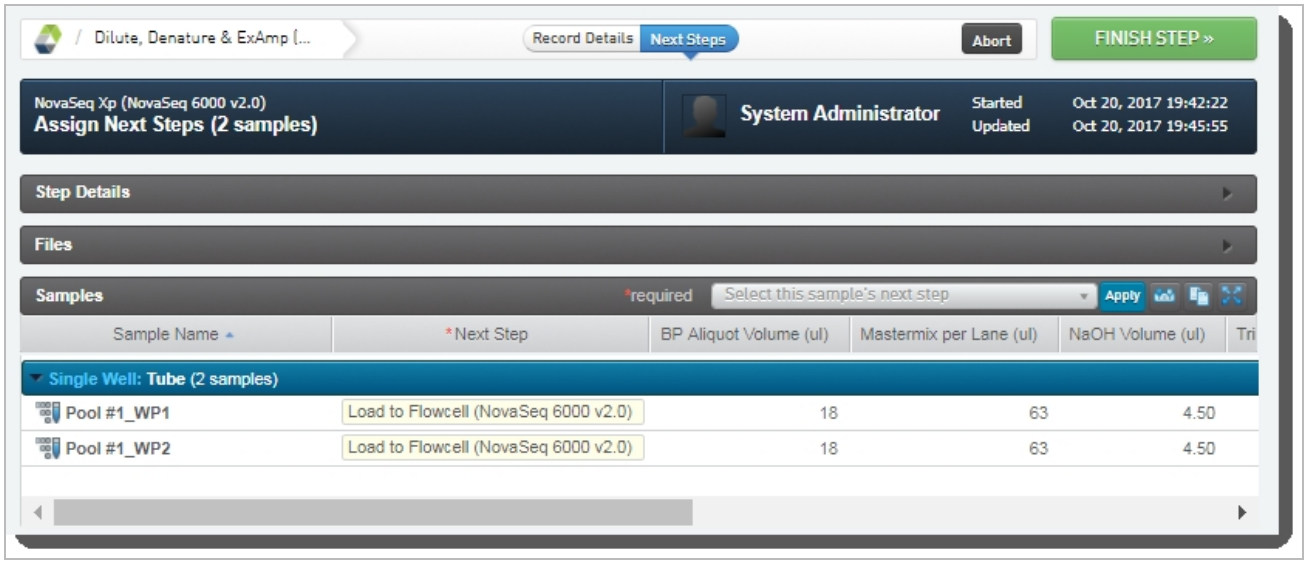


- In the Files area, the user can click on the Calculation File (CSV) file to download it and then open it in Excel to view information about the DPX Mastermix volume, and the volume of Mastermix, NaOH. and Tris-HCl to add per working pool.



- User clicks Next Steps, which triggers the Set Next Step automation (described above).

4. On the Assign Next Steps screen, the next step is already set to Load to Flowcell (NovaSeq 6000 v3.0). User clicks Finish Step.



Step 3: Load to Flowcell (NovaSeq 6000 v3.0)

In this step, the user scans the flow cell barcode into the LIMS, and then manually places the working pools into the lanes of the flow cell that will be used in the NovaSeq run. In addition, this step validates the run setup information and generates the sample sheet file.

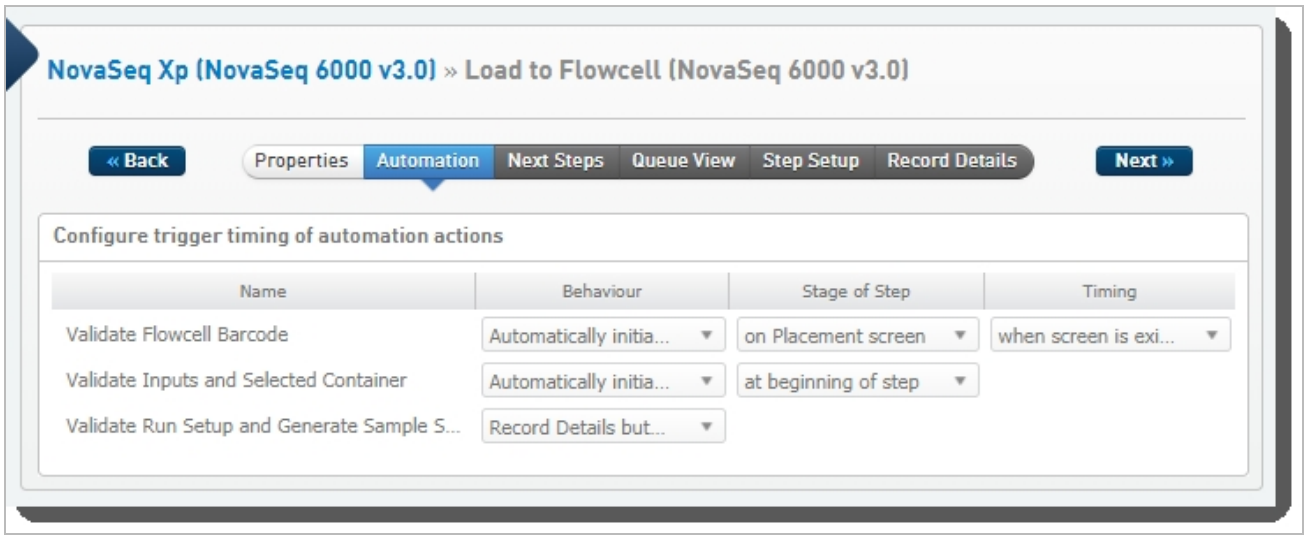
Step input: Working pool

Step output: Flow cell (output containers: SP, S1, and S2 with 2 lanes, and S4 with 4 lanes)

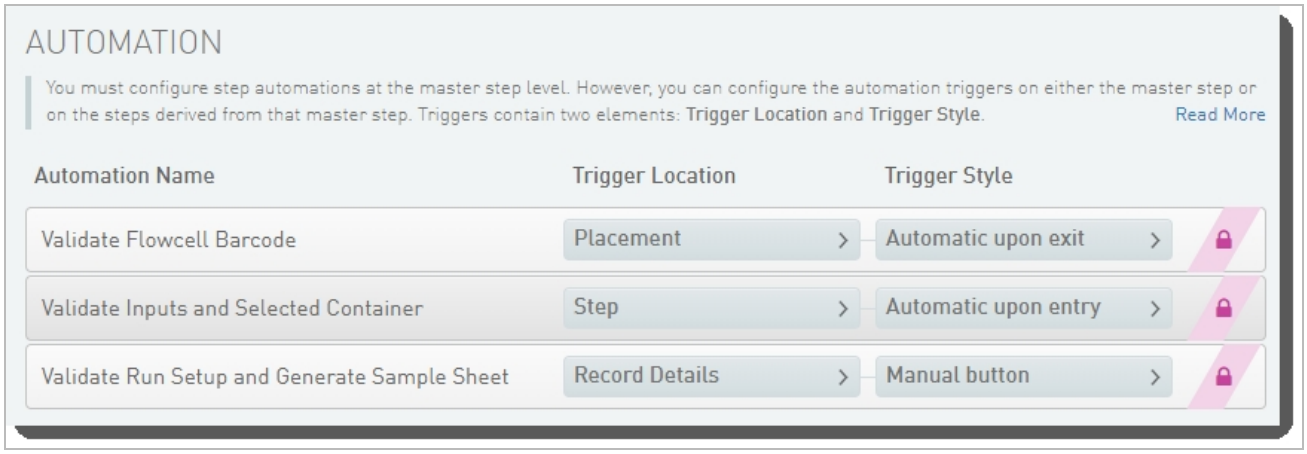
The following automations are configured on the step:

- Validate Run Setup and Generate Sample Sheet
- Validate Flowcell Barcode
- Validate Inputs and Selected Container

Load to Flowcell (NovaSeq 6000 v3.0) automation configuration - LIMS v4.2.x:



Load to Flowcell (NovaSeq 6000 v3.0) automation configuration - LIMS v5.x:



These automations are described below, in the order in which they are triggered at run time.

Validate Inputs and Selected Container

Automatically triggered at the beginning of the step, this automation invokes the validate_flowcell_for_input_pools and validateSelectedContainer scripts. These scripts validate the step inputs and the container selected by the user, as follows:

- Check that the Flowcell Type field has been set to a valid value (SP, S1, S2, or S4), and that all inputs have the same value.
- Check that the number of outputs matches the number of lanes on the selected flow cell type. If validation fails, an error message informs the user that the number of working pools does not match the number of lanes available on the flow cell.
  The following table defines the number of samples allowed:

Flow Cell Type	Number of Working Pools
SP	2
S1	2
S2	2
S4	4

- Check that the container type selected on entry to the Placement screen matches the value in the Flowcell Type field. If validation fails, displays an error message.

```
bash -l -c "/opt/gls/clarity/bin/java \
-jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar
\script:validate_flowcell_for_input_pools \
  -i {stepURI:v2} \
  -u {username} \
  -p {password} \
  -l {compoundOutputFileLuid1} \
  -validateSelectedContainer true"
```

Validate Flowcell Barcode

Automatically triggered on exit of the Placement screen, this automation:

- Validates the flow cell barcode scanned into the LIMS by the user, using the following logic:

```
if (input.::Flowcell Type:: == ::SP:: && !output.container.name.matches( ::[A-Za-
z0-9]{5}DRXX:: ) ) { \
  fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: ) \
} ; \
if (input.::Flowcell Type:: == ::S1:: && !output.container.name.matches( ::[A-Za-
z0-9]{5}DRXX:: ) ) { \
  fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: ) \
} ; \
if (input.::Flowcell Type:: == ::S2:: && !output.container.name.matches( ::[A-Za-
z0-9]{5}DMXX:: ) ) { \
  fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: ) \
} ; \
if (input.::Flowcell Type:: == ::S4:: && !output.container.name.matches( ::[A-Za-
z0-9]{5}DSXX:: ) ) { \
  fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: ) \
} ;
```

- Copies the Flowcell Type and Loading Workflow Type field values from step inputs to outputs:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ; \
output.::Loading Workflow Type:: = input.::Loading Workflow Type::'
```


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Validate Run Setup and Generate Sample Sheet

Automatically triggered when user clicks a button on the Record Details screen, this automation:

- Copies the Flowcell Type from the step input to the Run Mode field (hidden):

```
step.::Run Mode:: = input.::Flowcell Type::
```

- Validates the parameters entered on the Record Details screen, which will be used to set up the run and generate the sample sheet file. (See *Respond to Validation/Recipe Request Call from Sequencer* on page 863 section, below.)
  - Experiment Name may only contain alphanumeric, dash, or underscore characters. Spaces are not permitted.
  - When Workflow Type = No Index, Index Read 1 must be zero. For any other Workflow Type, Index Read 1 must be greater than zero.

```
if(!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) { \
  fail(::Experiment Name contains prohibited characters. Allowed characters are:
a-z, A-Z, 0-9, -, and _::) \
}; \
if(step.::Workflow Type::== ::No Index::) { \
  if(step.::Index Read 1::!= 0) { \
    fail(::Index Read 1 must be 0 if the Workflow Type is No Index.::) \
  } \
} else { \
  if(step.::Index Read 1::== 0) { \
    fail(::Index Read 1 must be greater than 0 if the Workflow Type is ::+
step.::Workflow Type::+ ::.::) \
  } \
};
```

- Checks the Paired End and Read 2 Cycles field values.
  - If Paired End = False, sets Read 2 Cycles value to 0.
  - If Paired End= True and 2 Cycles value is 0, generates an error.

```
if (!step.::Paired End::.toBoolean()) {
  step.::Read 2 Cycles:: = 0 \
}; \
if (step.::Paired End::.toBoolean() && step.::Read 2 Cycles:: == 0) \{ \
  fail(::Read 2 Cycles must not be zero if it is Paired End read.::) \
};
```

- Checks the Workflow Type and Read 2 Cycles field values.
  - If Workflow Type is set to Dual Index and Index Read 2 value is 0, generates an error.
  - If Workflow Type is set to No Index or Single Index, sets Index Read 2 to 0.

```
if (step.::Workflow Type:: == ::Dual Index:: && step.::Index Read 2:: == 0) {
```

```
  fail(::Index Read 2 must not be zero if Workflow Type is Dual Index.::)
};
if (step.::Workflow Type:: == ::No Index:: || step.::Workflow Type:: == ::Single
Index::) {
  step.::Index Read 2:: = 0
};
```

- Copies the Flowcell Type value from the step inputs to the step outputs:

```
output.::Flowcell Type::= input.::Flowcell Type::;
```

- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol - AUTOMATED - Run (NovaSeq 6000) v3.0.

```
nextStep = ::ADVANCE::
```

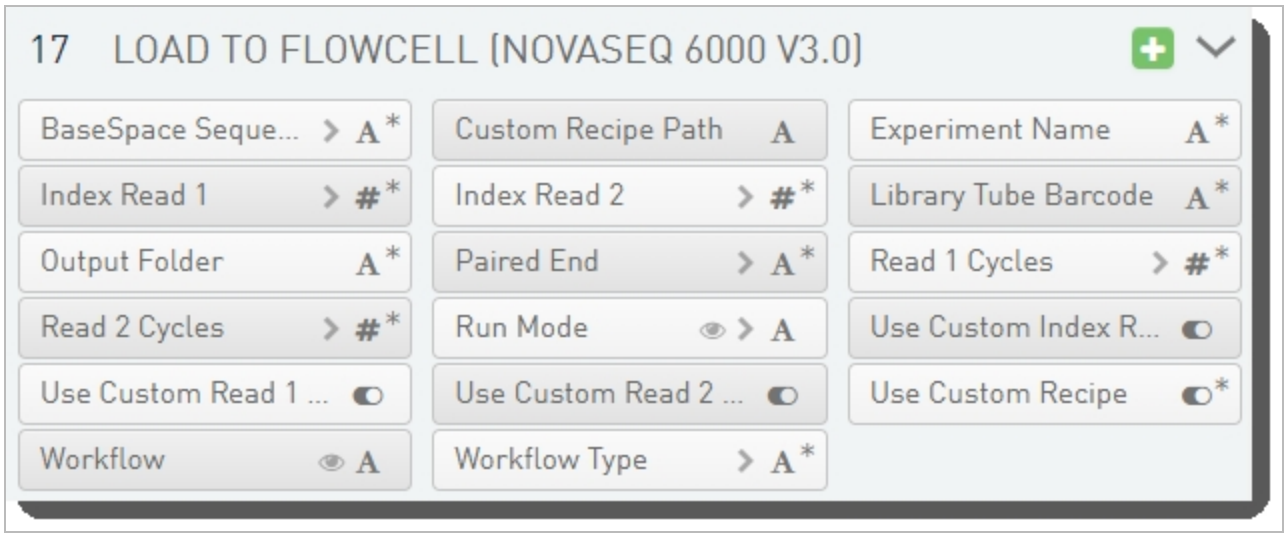
- Generates the sample sheet and attaches it to the step (see *Sample Sheet Generation* on page 861, below).

```
&& /opt/gls/clarity/bin/java \
-jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/DriverFileGenerator.jar \
  -i {stepURI:v2} \
  -u {username} \
  -p {password} \
script:driver_file_generator \
  -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NovaSeq_BCL2FASTQ_
Samplesheet.csv \
  -o {compoundOutputFileLuid0}.csv \
  -q true \
  -destLIMSID {compoundOutputFileLuid0} \
-l {compoundOutputFileLuid1}"
```

Step UDFs/Custom Fields

There are 17 fields defined on the Load to Flowcell (NovaSeq 6000 v3.0) step.

These are shown below in the BaseSpace Clarity LIMS v5.x Configuration area (Custom Fields > Master Step Fields tab).



See the following table for LIMS v4.2.x and LIMS v5.x field configuration details:

Field Name	Field Type	Field Contraints/Options	Preset Values/Additional Options and Dropdown Items
BaseSpace Sequence Hub Configuration	Check Box (v4.2.x) Toggle Switch (v5.x)	• Required: Np • Read Only: No	• Default: None Set
Use Custom Recipe	Check Box (v4.2.x) Toggle Switch (v5.x)	• Required: Yes • Read Only: No	• Default: No
Workflow	Single-line Text (v4.2.x) Text (v5.x)	• Required: No • Read Only: Yes	• GenerateFASTQ
Workflow Type	Single-line Text (v4.2.x) Text Dropdown (v5.x)	• Required: Yes • Read Only: No • Custom Entries: No	• No Index • Single Index • Dual Index • Custom

Global UDFs/Custom Fields

The following table lists the global UDFs/custom fields that are configured to display on the Load to Flowcell (NovaSeq 6000 v3.0) step.

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Configured on Analyte in LIMS v4.2.x and Derived Sample in LIMS v5.x:			

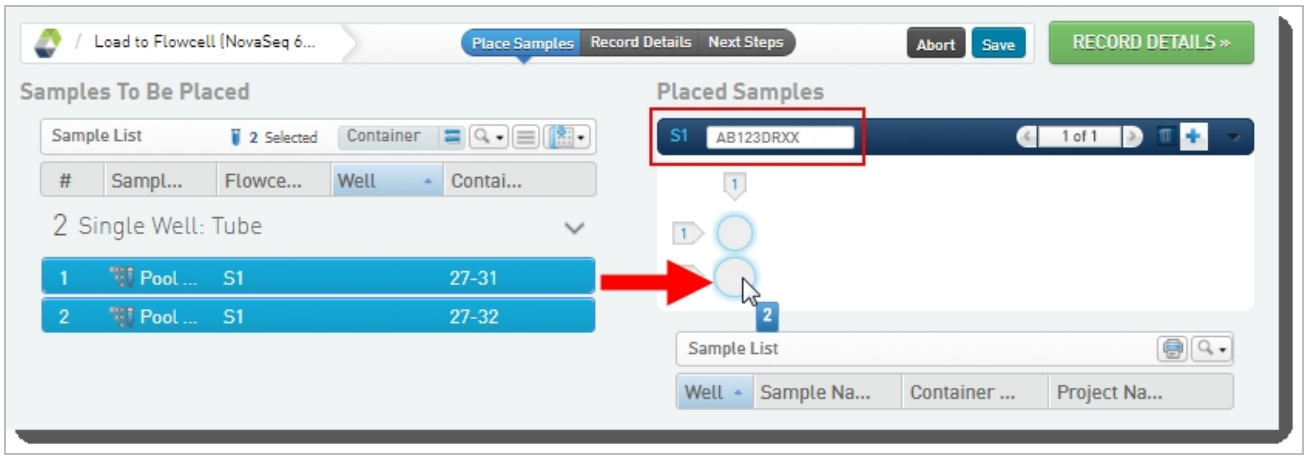
Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Flowcell Type	Single-line Text (v4.2.x) Text Dropdown (v5.x)	• Required: Yes • Read Only: No • Custom Entries: No	• SP • S1 • S2 • S4
Loading Workflow Type	Single-line Text (v4.2.x) Text Dropdown (v5.x)	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User Interaction

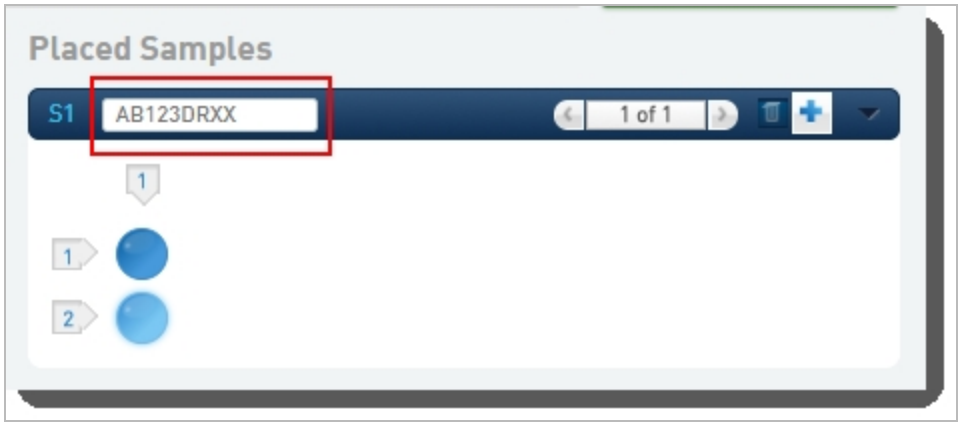
1. On the Ice Bucket screen, user:
    - Selects the appropriate flow cell type from the Destination Container drop-down list in the Container Options panel.



    - Clicks Begin Work.
2. On the Placement screen, user:
    - Drags the pool(s) from the left of the screen over into the Placed Samples area on the right.



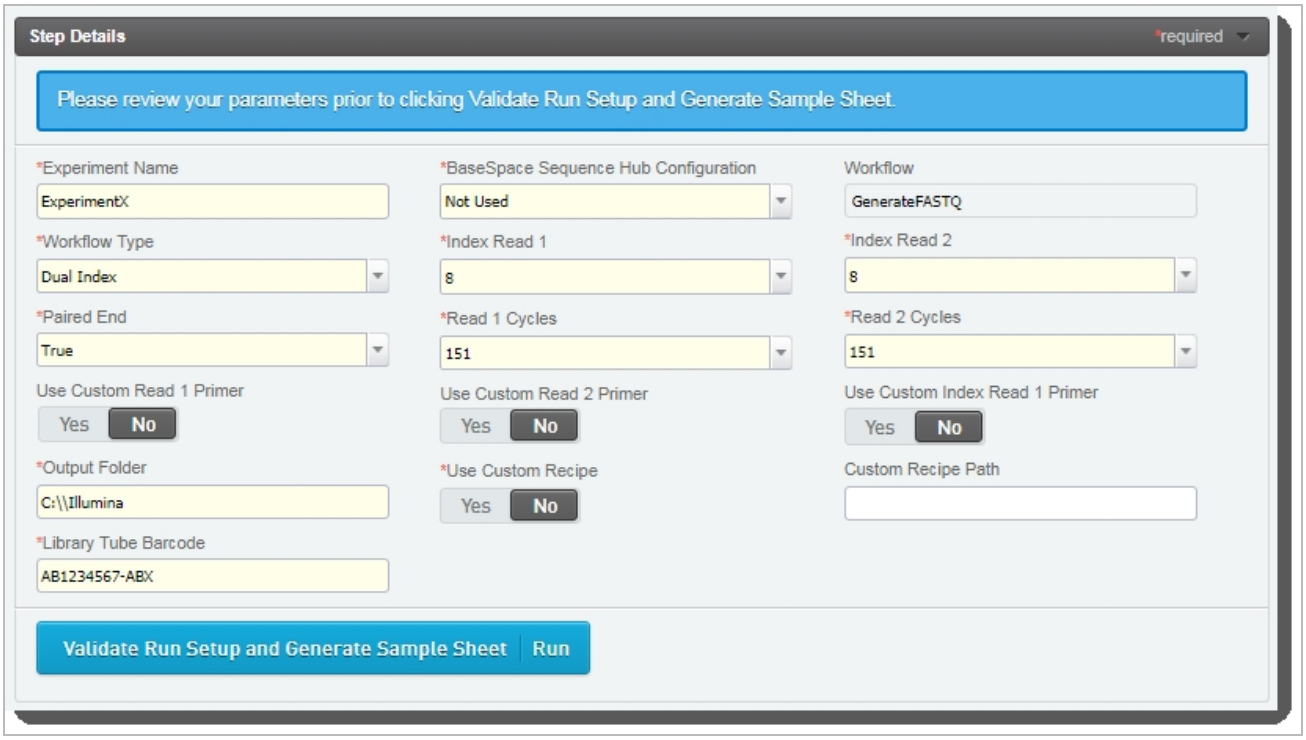
- Scans or types the barcode of the flow cell into the flow cell field.



- Clicks Record Details.

  On exit of the Placement screen, the Validate Flowcell Barcode automation validates the container barcode (described above).

3. The fields displayed on the Record Details screen are used to set up the run and generate the sample sheet file.

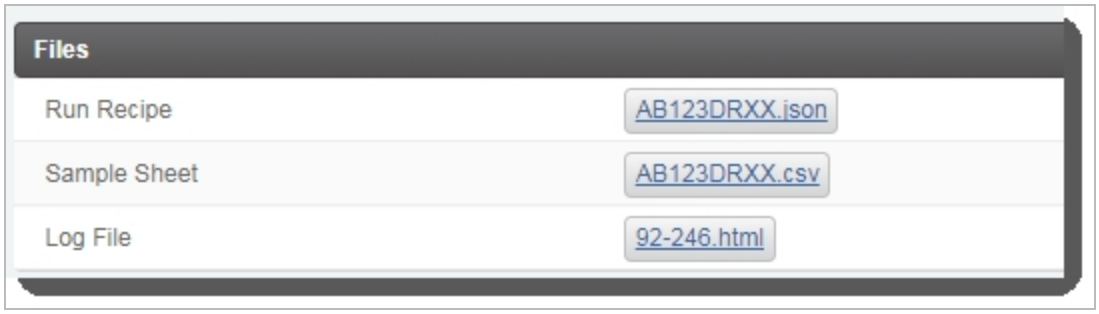


Some of these fields are auto-populated and some must be completed by the user (see the following table for details):

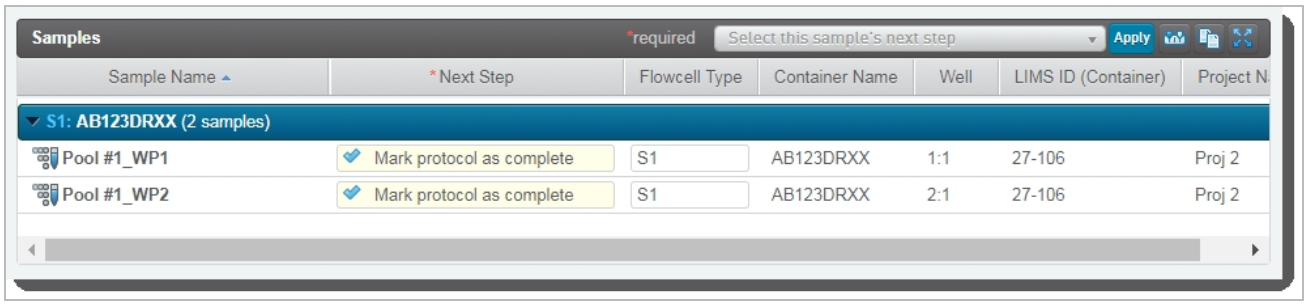
Field	Value
Experiment Name	User enters the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.
BaseSpace Sequence Hub Configuration	User selects from preset options: Not Used, Run Monitoring Only, Run Monitoring and Storage
Workflow	Automatically populated with preset - GenerateFASTQ
Workflow Type	User selects from preset options: No Index, Single Index, Dual Index, Custom
Index Read 1	User selects from preset options: 0, 6, 8 - or type a value between 0 and 20
Index Read 2	User selects from preset options: 0, 6, 8 - or type a value between 0 and 20
Paired End	User selects from preset options: True or False
Read 1 Cycles	User selects from preset options: 251, 151, 101, 51 - or type a value between 1 and 251 Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.

Field	Value
Read 2 Cycles	User selects from preset options: 251, 151, 101, 51 - or type a value between 1 and 251 Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Use Custom Read 1 Primer	User selects if applicable.
Use Custom Read 2 Primer	User selects if applicable.
Use Custom Index Read 1 Primer	User selects if applicable.
Output Folder	User enters the network path for sequencing run folder. For example: \\networkshare\run_data
Use Custom Recipe	User selects if applicable.
Custom Recipe Path	If Use Custom Recipe selected, user enters the path to the custom recipe file to be used.
Library Tube Barcode	User scans the library tube barcode.

4. On the Record Details screen, user clicks Validate Run Setup and Generate Sample Sheet. This triggers the automation script, which does the following:
    - Validates the information provided to set up the run.
    - Generates the sample sheet file and attaches it to the placeholder in the Files area of the Record Detailsscreen.



5. User clicks Next Steps.
6. On the Assign Next Steps screen, the Next Step field for all samples is pre-populated with Mark protocol as complete. User clicks Finish Step.



7. In Lab View, the pooled samples are queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0) step.

At this point in the workflow, the user interaction ends. The integration automatically starts the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0) step and data from the run is parsed back into the LIMS.

Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0)

This final protocol contains one fully-automated step, AUTOMATED- NovaSeq Run (NovaSeq 6000 v3.0), described below.

> This is a fully-automated step. The user must not add samples to the Ice Bucket or start the step manually.

Step 1: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0)

Step input: Library tube from NovaSeq Standard or flow cell from NovaSeq Xp protocol

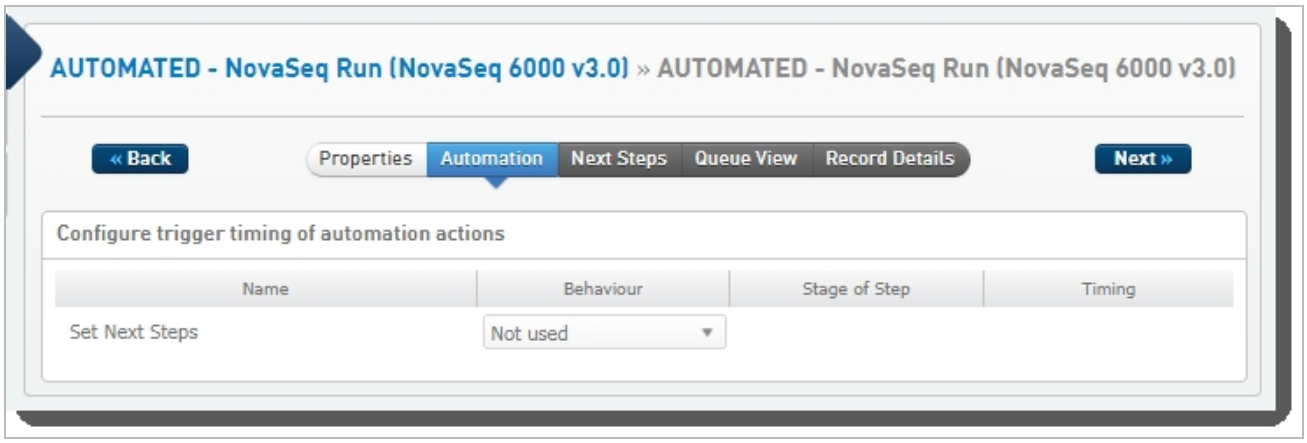
Step output: Result file/measurement

> The user must not start this step. This is a fully-automated step and the sequencing service will not update samples correctly if they have been manually started.

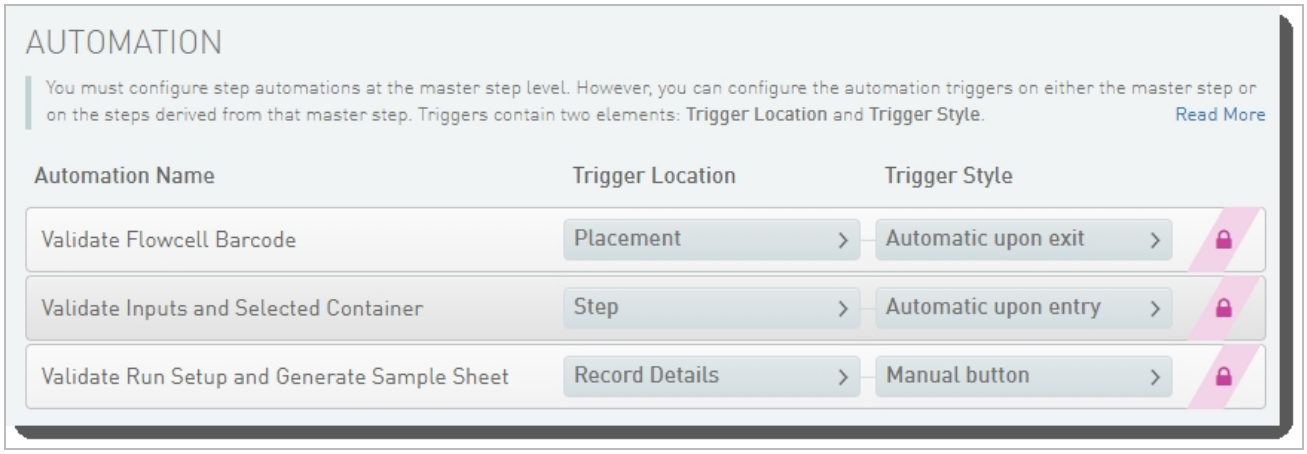
In this step, pooled samples are sequenced on the NovaSeq instrument and the run metrics are recorded in the LIMS.

The Set Next Steps automation is the only automation configured on this step. By default, this automation is disabled (set to Not Used) and the Sequencer API is used to set the next step for samples.

AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0) automation configuration - LIMS v4.2.x:



AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0) automation configuration - LIMS v5.x:



If required, you can override the default next step behavior used by the Sequencer API. To do this:

1. Enable the automation and use it to set custom next step behavior and generate a log file. By default, the automation will set the next step to ADVANCE and the samples complete the protocol.
2. Configure the automation to be triggered automatically on exit of the Record Details screen.

- If you do not want to override the default next step behavior used by the Sequencer API, you can do one of the following:
  - Leave the Set Next Steps automation in place, but disabled at the step level - as configured in the out-of-the-box integration.
  - Delete the Set Next Steps automation.
- If you would like to override the default next step behavior used by the Sequencer API, you can do one of the following:
  - Enable the automation and use it to set custom next step behavior and generate a log file - as described above.
  - Disable/configure auto-complete behavior by setting the values of the run.autoComplete and autoCompleteOnlyAtSuccess properties. See *Configuring autoComplete Properties* on page 874 section, below.

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Step UDFs/Custom Fields

The following table shows how the run parameters parsed from the RunParameter.xml file map to step UDFs/custom fields configured on the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0) step in the LIMS.

Run Parameter	Step UDF/Custom Field Name
currentCycle	Current Cycle
currentRead	Current Read
firmwareVersion	Firmware Version
FlowCellExpirationDate	Flow Cell Expiration Date
FlowCellId	Flow Cell ID
FlowCellLotNumber	Flow Cell Lot Number
FlowCellMode	Flow Cell Mode
FlowCellPartNumber	Flow Cell Part Number
FlowCellSide	Flow Cell Side
controlSoftwareVersion	Instrument Control Software Version
instrumentId	Instrument ID
instrumentType	Instrument Type
WorkflowType	Loading Workflow Type
OutputRunFolder	Output Folder
rtaVersion	RTA Version
RunId	Run ID
runStatus	Run Status
sequencingLog	Sequencing Log

Global UDFs/Custom Fields

The following global UDFs/custom fields are used to capture the run metrics in the LIMS.

- % Aligned R1
- % Aligned R2
- % Bases >=Q30 R1
- % Bases >=Q30 R2
- % Error Rate R1
- % Error Rate R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1

- % Prephasing R2
- %PF R1
- %PF R2
- Cluster Density (K/mm^2) R1
- Cluster Density (K/mm^2) R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- Reads PF (M) R1
- Reads PF (M) R2
- Yield PF (Gb) R1
- Yield PF (Gb) R2

Note the following:

- Values are aggregated across all lanes. Some values - for example Yield PF (Gb) R1 - are summed, while others are averaged.
- The names listed above are the default UDF/global field names installed with the NovaSeq Integration v3.2.1 configuration provided in the Preconfigured Workflow Package (PCWP) v1.2 or later.
- All UDFs/global fields are configured on the Container entity.
- All field names are configurable via the LIMS Configuration > Custom Fields screen (Global Fields tab).
- If you change the field names in the LIMS, you must also change them through the run.metricUdfNames properties file in the application.yml file for the Sequencer API (see *application.yml Properties* on page 868). All fields are configured to be visible on the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0) step's Record Details screen, in the Sample Details table.



- As these UDFs/fields are configured on the Container entity, they are visible on each input. However, they should not be confused with per-lane metrics.
- All run metrics tracked in the LIMS are overall metrics for the run; there are no per-lane metrics provided by NovaSeq at this time.

How the Sequencer API Integration Works

Sample Sheet Generation

The sample sheet is generated on the step prior to the run – Dilute and Denature (NovaSeq 6000 v3.0) in the NovaSeq Standard protocol or Load to Flowcell (NovaSeq 6000 v3.0) in the NovaSeq Xp protocol. This is the step where samples are placed on the library tube or flow cell that will be loaded in the NovaSeq instrument.

In the out-of-the-box configuration, the *Validate Run Setup and Generate Sample Sheet* on page 851 automation generates one CSV format sample sheet file for use with bcl2fastq v2.20 downstream analysis.

How it Works

The sample sheet is uploaded to the NVCS via the /Illumina/Sequencer/v2/sequencing-run/files endpoint to the Sequencer API. The files endpoint allows for a file to be downloaded from the LIMS using OAuth (instead of Basic Authentication), which is required for NovaSeq.

The run recipe response sets the sample sheet URL to the link to download the file from this endpoint, and sets the sampleSheetRequiresOAuth value to true.

Sample Sheet Contents

The sample sheet content is controlled by the following step UDFs/master step fields that display in the Step Details area of the Record Details screen.

- Experiment Name
- Read 1 Cycles
- Read 2 Cycles
- Workflow

See the *Bcl2fastq2 Sample Sheet Generation* article for detailed information on the following:

- Sample sheet generation script parameters and usage
- Sample sheet data and configuration options
- Enabling unique FASTQ file names per sequencing run

NovaSeq Run

The steps below outline the sequence of events that occurs when a flow cell is loaded onto the NovaSeq instrument.

The LIMS tracks per-instrument sequencing runs by appending the instrument name to the Run ID field value.

If the InterOp data does not include an InstrumentName value, capturing of run metrics proceeds and a warning is logged. If the InterOp data does include an InstrumentName value, and multiple instruments with the same name exist in the LIMS, capturing of run metrics fails and an error is logged.

User Authentication and Login

User authentication and login to the NovaSeq instrument is achieved via the getSequencerLoginURL endpoint (found at /Illumina/Sequencer/v2/sequencing-run/login). This endpoint returns the URL that the sequencer uses to get an access token.

1. The Sequencer API service provides the NovaSeq Control Software (NVCS) with a URL for the LIMS login screen and the NVCS displays this screen to the user. When the user logs in, this automatically authorizes the NVCS to use their account to access the LIMS.
2. User enters credentials:
    - Username and password are passed to the Clarity LIMS server, to ensure the access is valid.
    - By default, the Sequencer API service tries to contact the LIMS at http://localhost:9080/clarity/. This URL is set by the installation script, but can be configured with the clarity.url property in the application.yml file (see *application.yml Properties* on page 868).
3. User is redirected to the Sequencer login page at /Illumina/Sequencer/v2/sequencer_login.
    - If authentication is successful, the screen displays a success message. The LIMS server provides the OAuth token and username by returning them in redirect to /Illumina/Sequencer/v2/sequencer_login/Success. This redirect does not reload the page, but allows the NovaSeq to continue with run setup.
    - If authentication fails, the user is left on the login screen and a warning message informs them of the failure. A background redirect to /Illumina/Sequencer/v2/sequencer_login/Error informs the NovaSeq of the failure.

Respond to Validation/Recipe Request Call from Sequencer

1. The sequencing-run/recipe/novaseq endpoint consumes a POST request with a LibraryContainerI, FlowCellId, and reagents information.
    - LibraryContainerId, FlowCellId, and reagents information are provided by the NovaSeq. LibraryContainerId is required.
    - LibraryContainerId and FlowCellId should match either the library tube ID or flow cell ID of the corresponding container(s) in the LIMS.
    - If there are no containers or multiple containers, an error is generated.
2. All populated containers whose name matches either the LibraryContainerId or FlowCellId are validated as follows:
    - All samples in the container must be queued for a sequencing run step.
    - All samples must be queued for only one sequencing run step.
    - All samples must be queued for the same sequencing run step.



You can configure the steps that are considered as 'sequencing run steps' in the novaseq.sequenceStepNames property in the application.yml file. The property is a list and each list entry must be the exact name of a sequencing step, not the name of the underlying master step/process type. See *application.yml Properties* on page 868.

3. Once a single container has passed validation, the run setup information (run recipe request) is returned to the NVCS via the API and the run recipe is generated.
   Note the following:

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- The run recipe information is read from fields configured on the parent process/master step of the samples in the container. The workflow must include recipe definition in that step.
- By default, the parent process/master step is the Dilute and Denature (NovaSeq 6000 v3.0) or Load to Flowcell (NovaSeq 6000 v3.0) step, depending on workflow.
- The default field values match the names of the fields in the NovaSeq v3.2.1 configuration included in PCWP. The field names are configurable through the recipe.udfNames.* properties. Each of these properties corresponds to a entry in the recipe and the value of a property defines the name of the field that is used to populate that entry in the recipe.
- It is assumed that this parent process/master step removes any illegal characters from the Experiment Name field.
- The LIMS download link to the sample sheet created on the run setup step is used for the SampleSheet value in the recipe. The name of the output of the sample sheet on the setup step can be configured through the recipe.sampleSheet.outputName property (Sample Sheet by default) in the application.yml (see *application.yml Properties* on page 868). If no matching output is found, or no file was attached to the output, then the value of the recipe.sampleSheet.notAvailableValue property (Not Available by default) is used in the recipe.

4. After the recipe is generated, it is returned as a *.json file in the response, in the form defined by the Swagger definition for the API.

Run Recipe Contents

The contents of the run recipe are controlled by the sample sheet contents and the step UDFs/master step fields, configured on the Dilute and Denature (NovaSeq 6000 v3.0) and Load to Flowcell (NovaSeq 6000 v3.0) steps:

Example of run recipe content is as follows:

```
{
  "run_name": "NVCS16027_SPrime_2x250",
  "run_mode": "S4",
  "workflow_type": "DualIndex",
  "librarytube_id": "NV0025867-LIB",
  "flowcell_id": "",
  "sample_loading_type": "NovaSeqStandard",
  "rehyb": false,
  "paired_end": true,
  "read1": 151,
  "read2": 151,
  "index_read1": 8,
  "index_read2": 8,
  "output_folder": "\\\\network_path\\run_data",
  "samplesheet": "<path to sample sheet on LIMS server>",
```


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```
  "require_samplesheet_authentication": true,
  "usecustomrecipe": false,
  "customRecipe": null,
  "use_basespace": false,
  "basespace_mode": null,
  "use_custom_read1_primer": false,
  "use_custom_read2_primer": false,
  "use_custom_index_read1_primer": false
}
```

Respond to Sequencing Started Call from Sequencer

1. Matching container for the run (library tube or flow cell) is found, and contents are validated to be queued for the correct step.

> An error is thrown and the step is not started if no matching container is found, or if the samples in the container are incorrectly queued. This validation is also done during the run recipe request immediately prior, so this error case should not be encountered.

2. Reagent kits that are configured for the Sequencer API are checked against the configuration for the step to be run:
    - If a kit is configured for the API but does not exist in LIMS, it is created.
    - If a kit exists, but is not enabled on the step to be run, the step configuration is updated to enable it
    - If a kit that is to be enabled is Archived, it is set to Active.
    - If a kit that is to be enabled is in Pending status, it is set to Active.
    - If any kits enabled on the step are not configured for the Sequencer API, a warning is logged and the step is unable to be completed by the API without user intervention.
    - If any kits provided in the recipe request are not configured for the Sequencer API, they are ignored and a warning is logged during lot tracking.
3. The number of replicates for the step is determined by the type of flow cell being used in the run.
    - For Standard mode, this is based on the number of lanes in the flow cell (this is stored as part of the Sequencer API configuration).
    - For XP mode, this is always one replicate, since there is already one input tracked for each lane.
4. The automated sequencing step is started.
5. Reagent lots are attached to the step.
    - The Flow Cell and Library Tube reagents in the request are ignored - information from these is tracked in the LIMS as containers and UDFs.
    - Any other reagents in the request are handled as follows:If the reagent is not configured to be tracked, a warning is logged and the reagent is ignored.

- Existing reagent lots are looked up by kit name (determined by Sequencer API configuration), name (the serial number in the request), and lot number (the lot number in the request).
- If one or more matching reagent lot exists in the LIMS, the newest one is used:
  - If the expiry date does not match the expiry in the request, or if the status is not set to Active, the lot is updated to have the correct expiry date and an Active status.
- Otherwise a new lot is created with:
  - Reagent Kit: Matching kit as determined from Sequencer API configuration and step 2 above.
  - Name: Serial number in request
  - Lot Number: Lot number in request
  - Expiry: Expiry date in request
  - Status: Active
- If any reagents were expected to be in the request but were not, these reagents are logged. This prevents the step from being completed without user intervention.

6. Step UDFs/master step fields are updated with the run information parsed from the request. See *Respond to Record InterOps Metrics Call from Sequencer* on page 866, below.
7. Any important events during this request are logged into the Sequencer API log file as well as in the Sequencing Log multiline UDF/field on the step (assuming the step was started successfully). See *Respond to Record InterOps Metrics Call from Sequencer* on page 866, below.

Respond to Record InterOps Metrics Call from Sequencer

1. When the metrics endpoint receives a POST, it:
   - Looks for a matching library tube or flow cell in the LIMS.
   - Find finds the in-progress sequencing step for that container.
2. When the step is found, the run metrics in the request are written to container UDFs/global fields in the LIMS.

Respond to Sequencing Completed Call from Sequencer

If the sequencing run was successful, after the metrics have been recorded, the API call for Sequencing Completed is received. Information is matched by Sequencing Container and the following occurs in the LIMS:

1. The Current Cycle, Current Read, and Status UDFs/master step fields are updated.
2. The multiline Sequencing Log UDF/master step field is updated.
3. A success message is recorded in the log file, at the info level:

```
Sequencing run with Run ID {runId} of Step '{step.name}' ({step.limsid})
completed successfully.
```

4. The LIMS waits for any automations that are triggered automatically as it advances the step.

5. By default, the LIMS assigns the next steps on the outputs to move them on to the first configured next step - or, if there are no next steps, to complete the protocol.

Respond to Sequencing Errored Call from Sequencer

If the sequencing run was unsuccessful, the API call for Sequencing Error is received. Information is matched by Sequencing Container and the following occurs in the LIMS:

1. The Current Cycle, Current Read, and Status UDFs/master step fields are updated.
2. The multiline Sequencing Log UDF/master step field is updated.
3. A failure message is recorded in the log file, at the error level:

```
Step '${Step.name}' (${Step.limsid}) failed with Run ID ${runId}.
```

4. The step is left at the Record Details screen.

Respond to Sequencing RunEndedByUser Call from Sequencer

If the user ends the sequencing run, the API call for Sequencing RunEndedByUser is received. Information is matched by Sequencing Container and the following occurs in the LIMS:

1. The Current Cycle, Current Read, and Status UDFs/master step fields are updated.
2. The multiline Sequencing Log UDF/master step field is updated.
3. The following message is recorded in the log file, at the warning level:

```
"Sequencing run with Run ID ${status.runInfo.runId} of Step
'${step.configuration.name}' (${step.limsid}) aborted by user
${status.runInfo.userName}."
```

4. The step is left at the Record Details screen.

Components Installed with this Integration

The following sections describe the various components - files, properties, reagent categories / label groups, reagent kits, and containers - that are installed by default as part of this integration. Information on installed workflows, protocols, steps, and automation points is provided in the Workflows, protocols, and steps section, above.

Illumina NovaSeq Integration v3.2.1 is distributed as an RPM package - BaseSpaceLIMS-sequencer-api. This RPM package must be installed on the LIMS server.

The BaseSpaceLIMS-sequencer-api RPM installs the following:

- Sequencer API WAR file
- Configuration file - *application.yml*
- Two configuration scripts - *configure_sequencer_api_proxy.sh* and *configure_sequencer_api_application.sh*

The NovaSeq v3 configuration is delivered through the Preconfigured Workflows Package (PCWP) v1.2, which requires installation of the NGS Extensions Package v5.18 or later.

- If the NGS Extensions Package is not already installed, or if a version earlier than v5.18 is installed, the latest version is installed by default with the NovaSeq integration. See Release Notes for details.
- If NGS Extensions Package v5.18 or later is already installed, upgrade is not forced or required.

The RPM package installs the components listed in the following table:

Files Installed	Location and Description
Illumina#Sequencer#v2.war	*/opt/gls/clarity/tomcat/current/webapps* War file for NovaSeq v3.2.1 API
configure_sequencer_api_application.sh	*/opt/gls/clarity/config/* Script that configures the Sequencer API application through its external application.yml file
configure_sequencer_api_proxy.sh	*/opt/gls/clarity/config/* Script that configures the proxy to allow communication with the Sequencer API (NovaSeq v3.2.1 API) application
sequencer-api.conf	*/etc/httpd/clarity/* proxy setting to communicate with the LIMS

application.yml Properties

The application.yml file is located at opt/gls/clarity/extensions/sequencing-api/.

- All properties are configured automatically during installation, but you can also configure them by editing the application.yml file and then restarting Tomcat.
- All custom field names, both for recipe and run information, are represented by properties in the file.
- For all custom fields, the names (values in this file) can be changed. However, the property names cannot be changed, removed, or added to. For example, you can change Loading Workflow Type but you cannot change recipe.udfNames.sampleLoadingType.

The properties it contains are listed in the following table:

Property	Description
spring.profiles.active	Tells the application that it is running deployed in Tomcat. Default value: "tomcat" DO NOT CHANGE
clarity.url	Base URL that the Sequencer API service uses to contact the LIMS. The installation script (configure_sequencer_api_application) prompts for this.
clarity.username	Username to be used when communicating with Clarity LIMS

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Property	Description
clarity.password	Encrypted password to be used when communicating with Clarity LIMS
security.signing-key	Private key that should be used when signing/validating OAuth tokens. Changing this will invalidate any issued tokens.
security.token-expiry	Specifies (in hours) the expiry period for login tokens issued by the Sequencer API. Default value: 88
novaseq.sequenceStepNames	List of NovaSeq sequencing run step names for which the integration may find samples queued. • Names must be an exact match to the name of the step in the LIMS (not the master step/process type). • Each line should be indented to the same point and start with a dash (-). Default value: `- "AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0)"`
novaseq.flowcells	List of supported NovaSeq flow cell types. • Type names exactly match the values used by the NVCS run status requests. • To add more types, provide both type and lanes properties, indented to the same point and starting with a dash (-). Default value: `- type: "SP"lanes: 2- type: "S1"lanes: 2- type: "S2"lanes: 2- type: "S4"lanes: 4`
novaseq.reagents	List of supported reagent kits to be tracked on sequencing steps. - lotName: The name of the lot as it appears in the run started request. - kitName: The name of the corresponding reagent kit in LIMS that the lot should be tracked as. • The name may differ in the NVCS request vs. the LIMS configuration. Default value: `- lotName: "Buffer"kitName: "Buffer Cartridge"- lotName: "Cluster"kitName: "Cluster Cartridge"- lotName: "SBS"kitName: "SBS Cartridge"`

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Property	Description
recipe.udfNames	Configures the names of the UDFs/fields that will be used in the run recipe (see *Run Recipe Contents* on page 864 for more information). • Each value must be enclosed in quotes. Example: recipe.udfNames.runMode "Run Mode" • Value provided for sampleLoadingType must match the value provided in the run configuration. Example: recipe.udfNames.sampleLoadingType "Loading Workflow Type"
recipe.sampleSheet.outputName recipe.sampleSheet.notAvailableValue	Configures the name of the sample sheet file placeholder on the step where run recipe information is populated, and the property value to use when the file is not found.
run.stepUdfNames	Configures the step UDF/master step field names that will be used when recording run results (see *Step UDFs/Custom Fields* on page 860 for more information). • Each value must be enclosed in quotes. Example: run.stepUdfNames.runID "Run ID" • Value provided for sampleLoadingType must match the value provided in the run configuration. • Example: run.stepUdfNames.sampleLoadingType "Loading Workflow Type". It is expected that the run step field and the field on the input derived sample will share the same name.
run.metricUdfNames	Configures the result file UDFs/measurement global fields names that will be used when recording run results. These are the fields that capture the run data (see *Global UDFs/Custom Fields* on page 860 for more information).
run.autoComplete	Determines whether the sequencing run step should be completed automatically after parsing the run metrics on a run (see also run.autoCompleteOnlyAtSuccess). Default value: true See *Configuring autoComplete Properties* on page 874, below.

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Property	Description
run.autoCompleteOnlyAtSuccess	Determines whether the sequencing run step should be: • Autocompleted when the run status is RunCompletedSuccessfully (ifautoCompleteOnlyAtSuccess is true) - or - • Autocompleted regardless of the run status (if autoCompleteOnlyAtSuccess is false). Default value: true See *Configuring autoComplete Properties* on page 874, below.

Reagent Categories/Label Groups

- TruSeq HT Adapters v2 (D7-D5)

Reagent Kits

- Buffer Cartridge
- Cluster Cartridge
- DPX1
- DPX2
- DPX3
- NaOH
- Resuspension Buffer (RSB)
- SBS Cartridge
- Tris HCl

Container Types

- Library Tube
- SP
- S1
- S2
- S4

This integration supports:

- Library tube with barcode provided in the format [A-Za-z]{2}[0-9]{7}-[A-Za-z]{3}
  Example: AB1234567-XYZ
- SP, S1, S2, or S4 flow cell, with barcode provided in one of the following formats:
  - [A-Za-z0-9]{5}DRXX
  - [A-Za-z0-9]{5}DMXX

- [A-Za-z0-9]{5}DSXX

  Example: AB123DRXX

NVCS Configuration

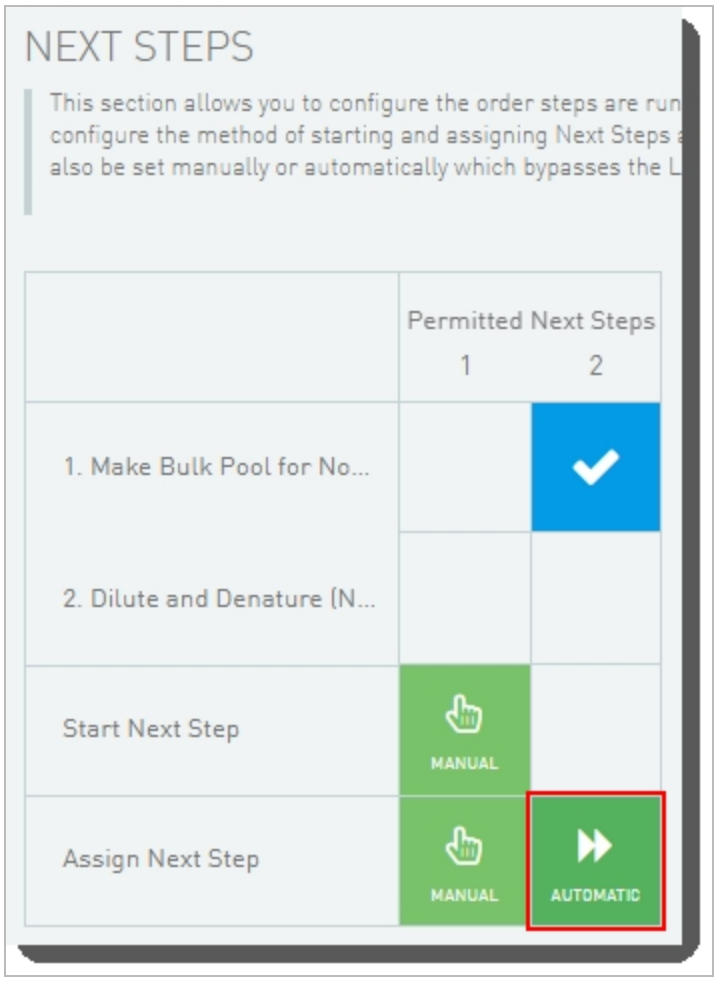
For details on configuring NVCS for integration with BaseSpace Clarity LIMS, contact the Clarity LIMS Support team.

Routing Script Requirements

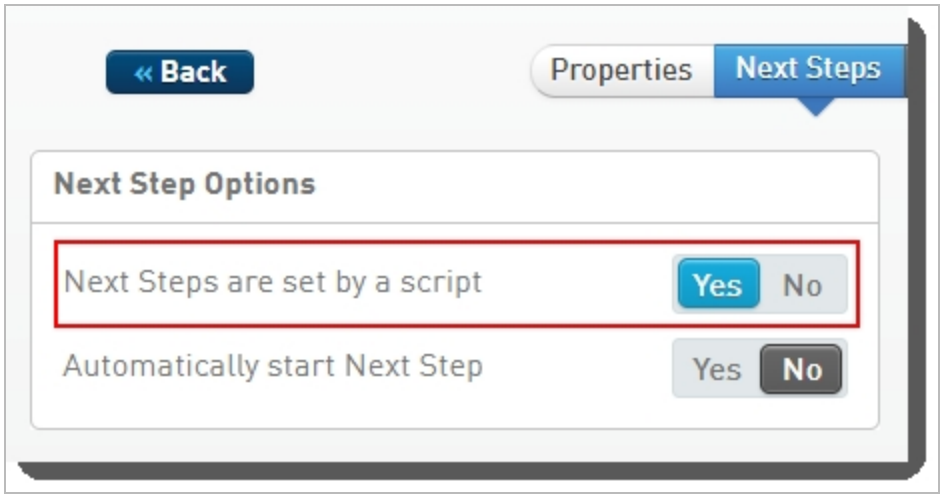
The requirements for the routing script functionality are as follows:

- On the steps that use the routing script - Define Run Format (NovaSeq 6000 v3.0) and Dilute and Denature (NovaSeq 6000 v3.0) - the Next Step for all samples must be set to Remove from workflow. This value is set by a script, and the user running the step must not change this value in the Assign Next Steps screen.
- LIMS v5.x: In the protocol configuration screen, the following settings are required and must not be changed:
  In the Next Steps section, for the last step in the protocol, the method of assigning the next step must be set to Automatic.

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- LIMS v4.2.x: In the protocol configuration area, for all protocol steps on which the Routing Script automation is configured, the following setting is required and must not be changed:
  On the Next Steps configuration tab, the Next Steps are set by a script toggle switch must be set to Yes.



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Configuring autoComplete Properties

As of NovaSeq Integration v3.2.1, the application.yml configuration file contains two additional properties:

- run.autoComplete
- run.autoCompleteOnlyAtSuccess

These properties determine the conditions under which the last step of the NovaSeq 6000 workflow - AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0) - is automatically completed:

- Only when the run status is "RunCompletedSuccessfully" (default behavior)
  -or -
- Regardless of the run status

By default, both properties are set to 'true' and the step only completes if the run is successful. If the run fails or is aborted by the user, the user must manually complete the step in the LIMS.

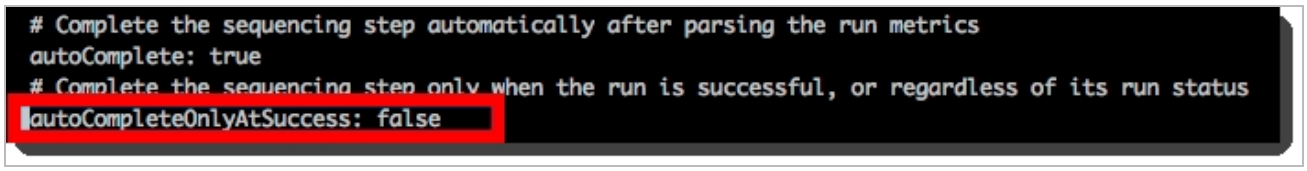
If you would like the step to autocomplete regardless of the result of the sequencing run, you must change the autoCompleteOnlyAtSuccess property value to 'false'.

Enabling/Disabling autoComplete Properties

1. Open the application.yml file located at /opt/gls/clarity/extensions/sequencer-api/application.yml:

```
vi /opt/gls/clarity/extensions/sequencer-api/application.yml
```

2. Edit the value of the autoCompleteOnlyAtSuccessproperty as required.



3. Save the file.

The following table shows how the combined value of the run.autoComplete and run.autoCompleteOnlyAtSuccess properties affects the auto-complete behavior of the sequencing step.

run.autoComplete property value	run.autoCompleteOnlyAtSuccess property value	Outcome
true (default)	true (default)	• Step auto-completes only if sequencing run is successful. • If run is not successful, step does not auto-complete and run details are recorded in the LIMS.
true	false	• Step auto-completes, regardless of the run status.

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run.autoComplete property value	run.autoCompleteOnlyAtSuccess property value	Outcome
false	true	• Step does not auto-complete.
false	false	• Run details are recorded in the LIMS.

Rules and Constraints

- The workflow configuration contains several validation checks. To ensure that the calculations work properly, it is important that you do not disable any of this validation logic. The validation checks determine:
  - Which samples, and how many, can enter each step together.
  - Which samples, and how many, can be pooled together.
- The library tube ID must be unique. There should not be multiple library tube containers in the system with the same name.
- Reagent labels (indexes) must be unique.
- Only controls are permitted as unindexed samples; all other unindexed samples and pools are not permitted.
- For sample sheet generation constraints, refer to the Bcl2fastq2 Sample Sheet Generation article.
- Users must not manually start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0) step. This is a fully-automated step and the sequencing service will not update samples correctly if they have been manually started.
- For the automated run to start successfully, the user must have clicked the Validate Run Setup and Generate Sample Sheet button.

Resources

- *NovaSeq 6000 Sequencing System Guide*

NovaSeq 6000 Integration v3.2.1 Validation and Troubleshooting

This guide explains how to validate the installation of the Illumina NovaSeq 6000 Integration Package v3.2.1.

The validation process involves:

- Running samples through the Library Prep Validation v1.0 workflow.

  - The workflow contains a single-step protocol that models the library prep required to produce normalized libraries. At the end of the step, the normalized libraries are advanced to the workflow selected by the user.
- Running normalized libraries through the NovaSeq 6000 v3.0 workflow. This validates the following:
  - Successful routing of samples from the Run Format (NovaSeq 6000 v3.0) step to the NovaSeq Standard (NovaSeq 6000 v3.0) or NovaSeq Xp (NovaSeq 6000 v3.0) step.
  - Automated generation of a sample sheet for use with bcl2fastq2 v2.20 analysis software. This file is automatically uploaded to the sequencing instrument via the Sequencer API and is used to set up the run.
  - Automatic validation of run setup information. This information is uploaded to the NovaSeq Control Software (NVCS) via the the Sequencer API and is used to create the run recipe and initiate the run.
  - Automated tracking of the NovaSeq sequencing run and parsing of run statistics into the LIMS, via the Sequencer API.

Before You Begin

Before you begin to run the validation steps below, note that these steps assume that you have installed the Illumina NovaSeq 6000 Integration Package v3.2.1 and have imported the default LIMS configuration.

Validation Steps

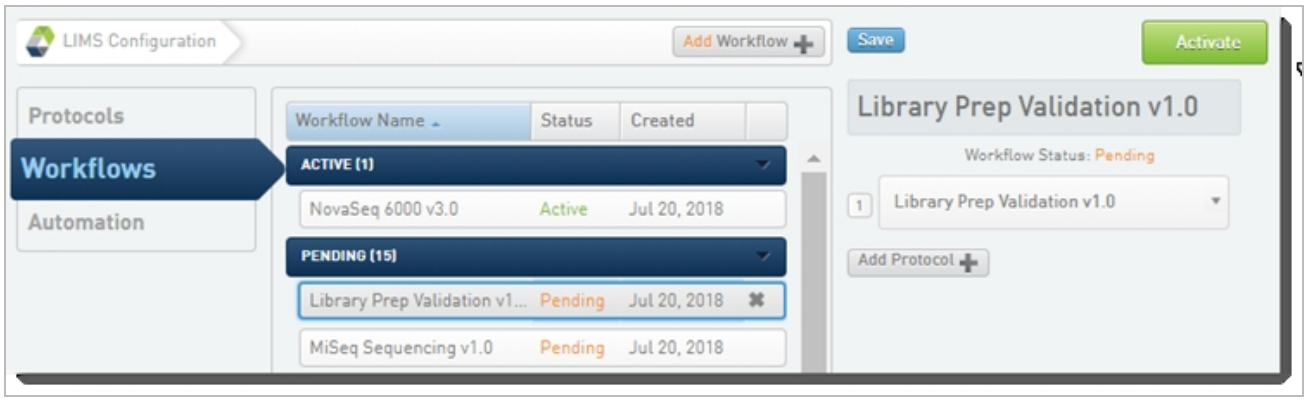
Activate Workflow, Create Project, Add and Assign Samples

The following steps set up the LIMS in preparation for running samples through the Library Prep Validation v1.0 and NovaSeq 6000 v3.0 workflows.

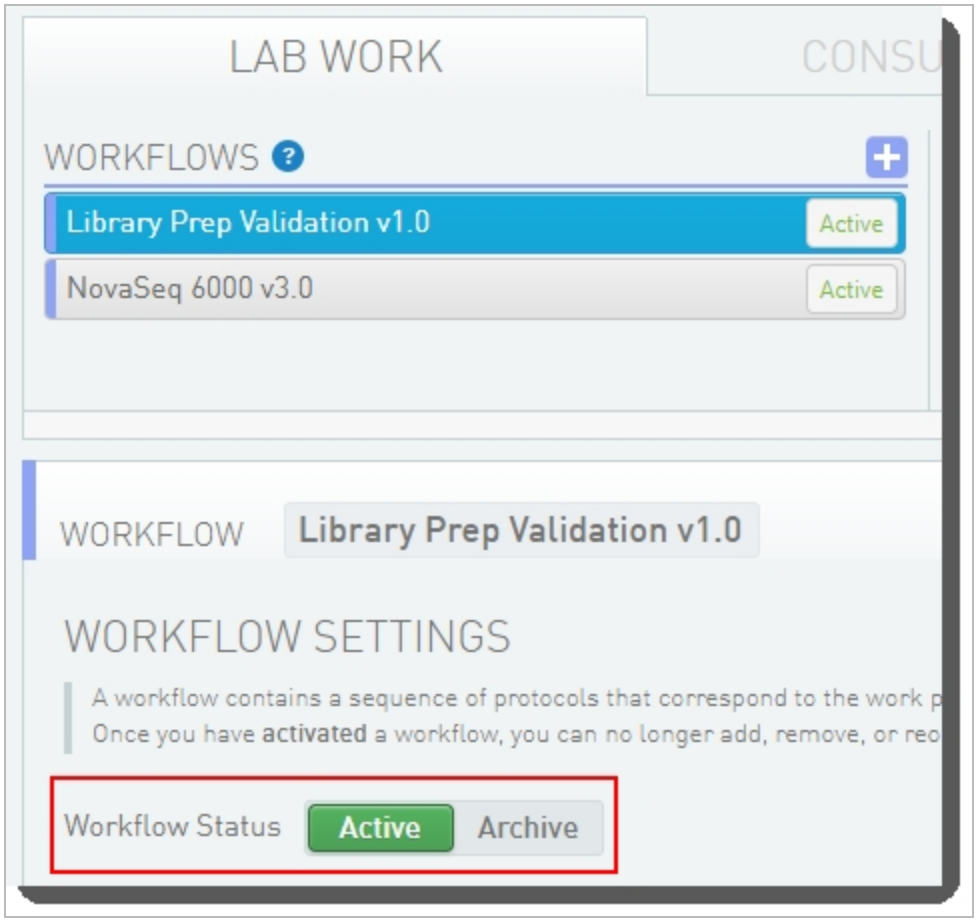
Step 1: Activate the Workflow

In the LIMS configuration area, activate the Library Prep Validation v1.0 and NovaSeq 6000 v3.0 workflows.

Workflow configuration and activation - LIMS v4.2:



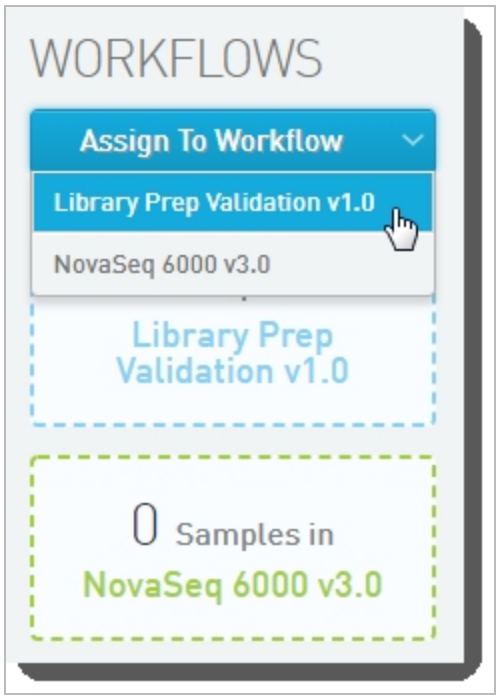
Workflow configuration and activation - LIMS v5:



Step 2: Create a Project, Add Samples

1. On the Projects and Samples screen, create a project and add samples to it.
2. Assign your samples to the Library Prep Validation v1.0 workflow.

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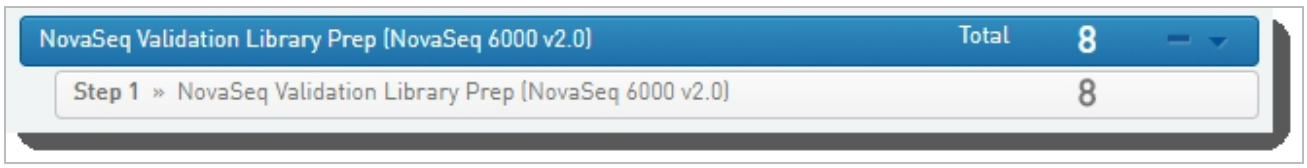


Library Prep Protocol: Library Prep Validation v1.0

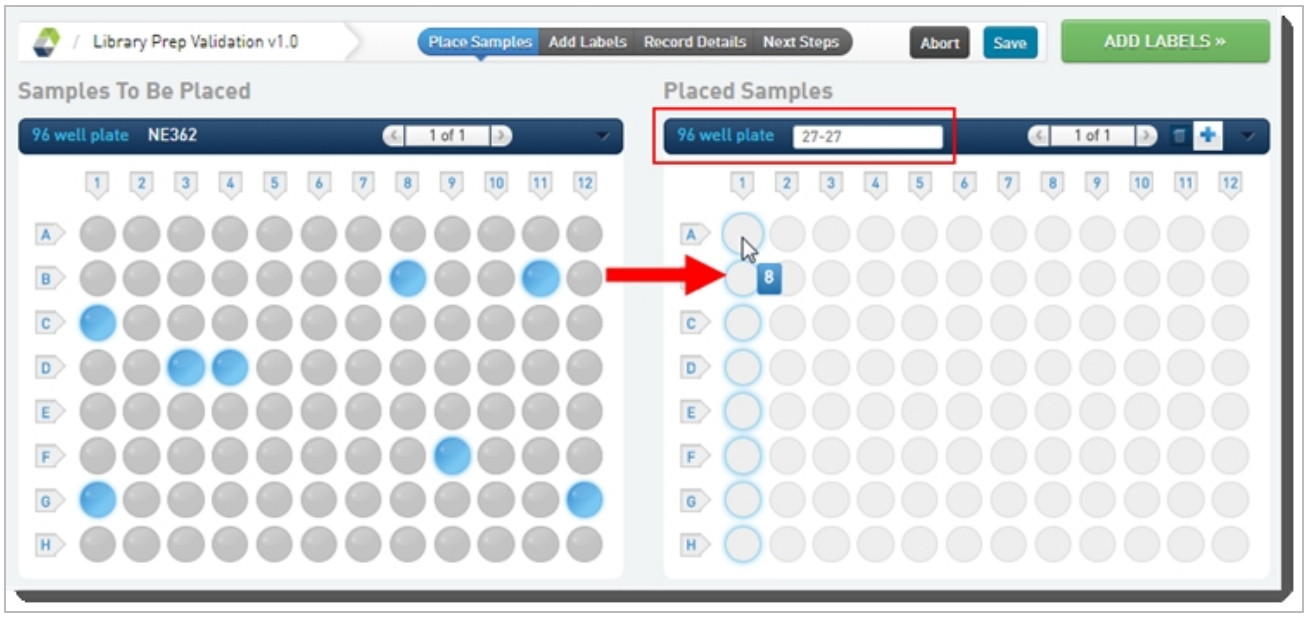
This single-step protocol models the library prep required to produce normalized libraries that are ready for the NovaSeq 6000 v3.0 workflow.

Step 1: Run Library Prep Validation v1.0

1. In Lab View, locate the Library Prep Validation v1.0 protocol. You will see your samples queued for the Library Prep Validation v1.0 step.



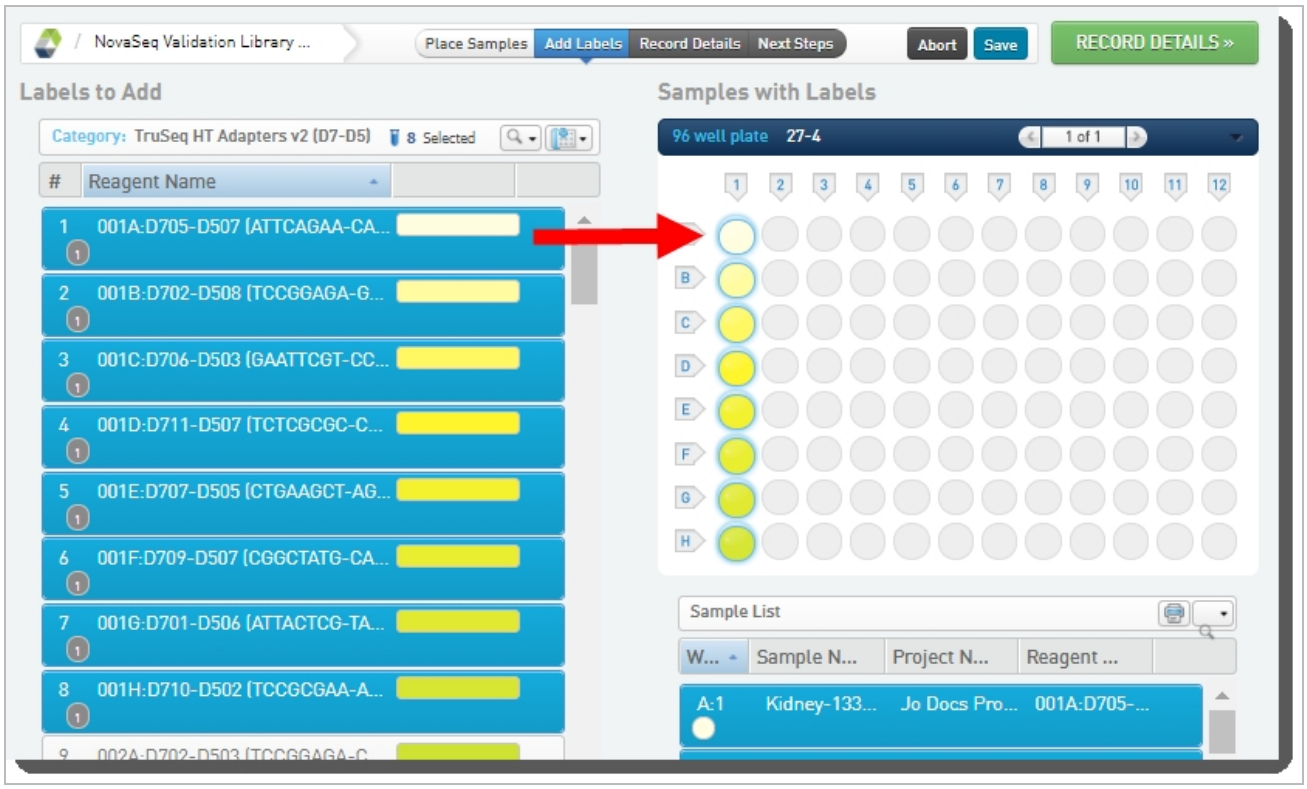
2. Add the samples to the Ice Bucket and click View Ice Bucket.
3. On the Ice Bucket screen, click Begin Work.
4. On the Placement screen:
    - In the Placed Samples area on the right, in the container field, scan or enter the barcode of the destination container.
    - Select the samples from the Samples to be Placed area on the left, and then drag them over to the container wells on the right.



- Click Add Labels.

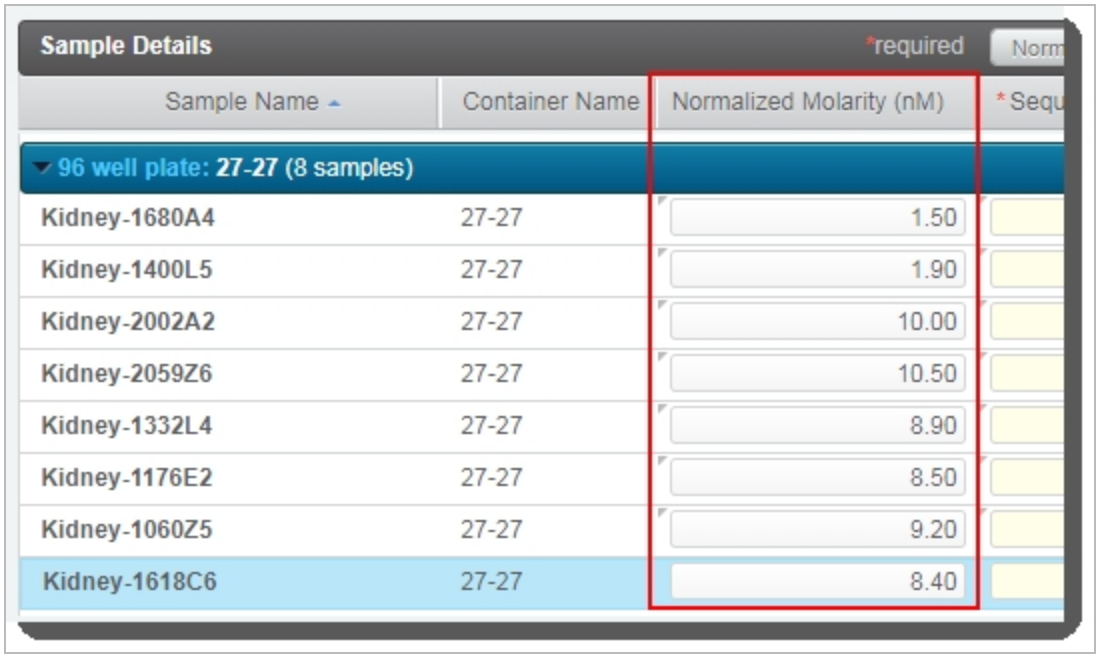
5. On the Add Labels screen, the Labels to Add list is populated with labels from the TruSeq HT Adapters v2 (D7-D5) label group. This is the only label group configured in the out-of-the-box integration and it is used by default in this step.
    - Select labels on the left, and drag them over to the container on the right to assign them to placed samples.

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- Click Record Details.

6. On the Record Details screen:
   - In the Sample Details table, enter values for Normalized Molarity. You can type values individually, or use Copy/Paste functionality to enter multiple values from a spreadsheet.

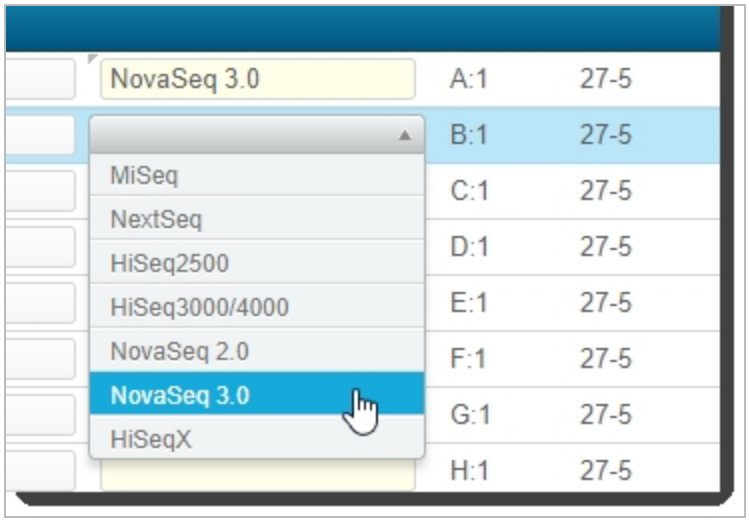


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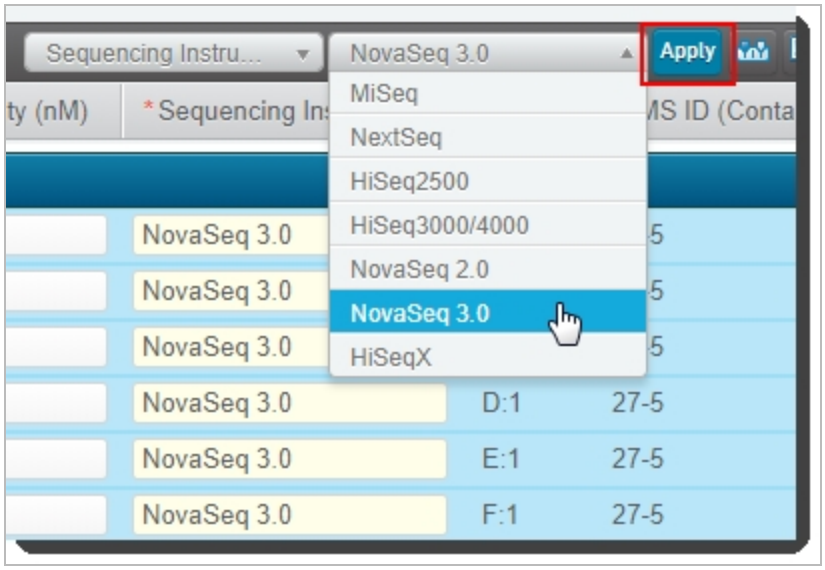
- The Sequencing Instrument column indicates the instrument to be used for the sequencing run. Select NovaSeq 3.0.

You can populate the fields in this column using one of the following methods:

- To populate fields individually for each sample: Select from the drop-down list in each row.



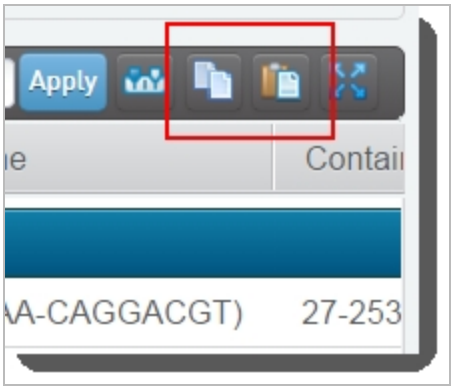
- To populate fields all at once: Use Copy/Paste to enter multiple values from a spreadsheet. Alternatively, click inside the table and press Ctrl + A to select all rows. At the top of the table, select Sequencing Instrument and then select NovaSeq 3.0 from the adjacent drop-down list. Click Apply.



If you are working with a large number of samples, you can use Copy/Paste functionality to enter multiple values from a spreadsheet.

a. Click the Copy Samples button to copy the sample information from the LIMS to the Clipboard.

b. Open a blank Excel document and paste the contents of the Clipboard into the spreadsheet.



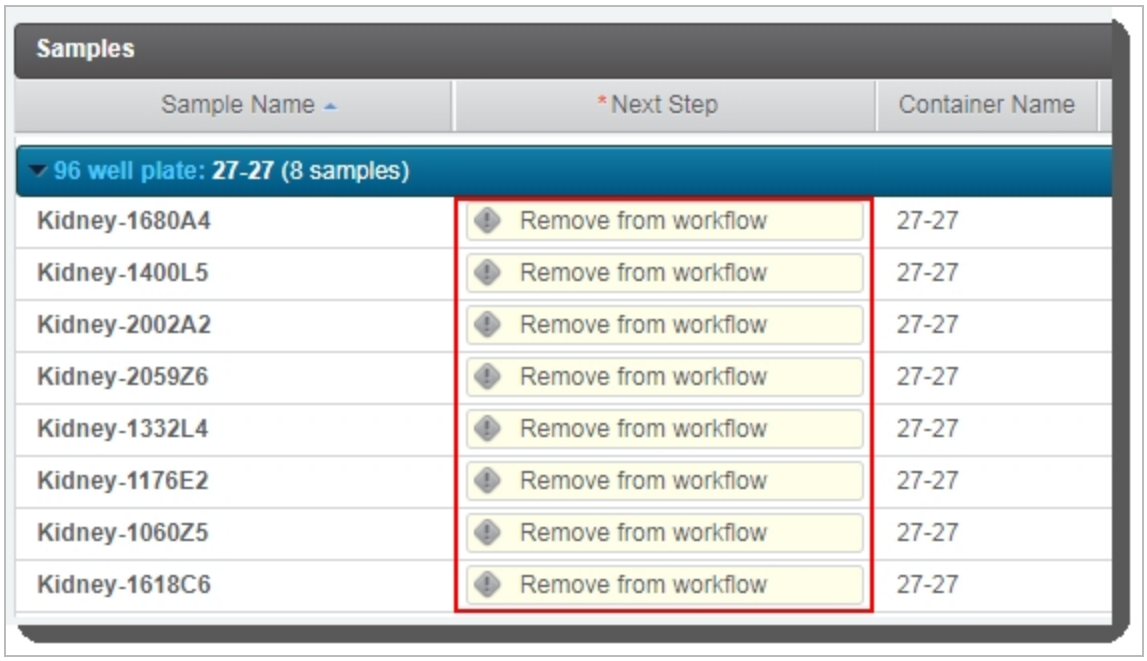
c. In the spreadsheet, enter the Normalized Molarity and Sequencing Instrument values. Select the entire spreadsheet, including the headers (Ctrl + A), and copy (Ctrl + C) the information to the Clipboard.

d. In the LIMS, click the Paste Samples button. Paste the contents of the clipboard (Ctrl + V) into the Update Table window and then click Update to paste into the Sample Details table.

7. Click Next Steps.
   - This triggers the Next Steps automation, which sets the value of the next step (for all samples) to Remove from workflow. Note that the Routing Script automation expects this value and requires it in order to successfully advance samples to the next step.
8. On the Assign Next Steps screen, in the Samples table, the Next Step for all samples is pre-populated with Remove from workflow.

Do not change this value! If Next Step is not set to Remove from workflow, the routing script will not be able to route samples correctly.

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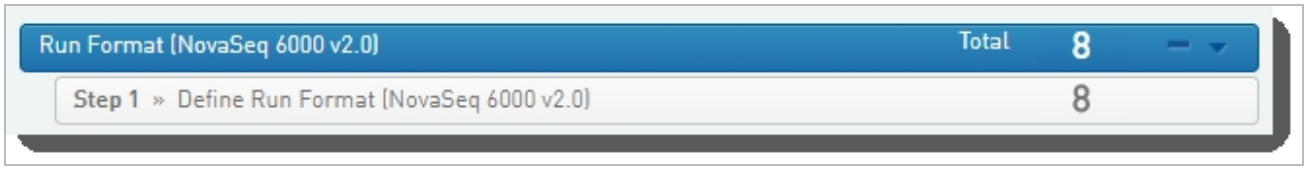
9. Click Finish Step.
   On exit from the step, the Routing Script automation is triggered. This automation assigns samples to the first step of the NovaSeq 6000 v3.0 workflow - Define Run Format (NovaSeq 6000 v3.0). This is the only step in Protocol 1: Run Format (NovaSeq 6000 v3.0).

Protocol 1: Run Format (NovaSeq 6000 v3.0)

This protocol includes a single step - Define Run Format (NovaSeq 6000 v3.0). The step allows for the assignment of per sample values for Loading Workflow Type, Normalized Molarity, Flowcell Type, and Final Loading Concentration (pM). At the end of the step, samples are routed to the NovaSeq Standard or NovaSeq Xp protocol, according to the selected Loading Workflow Type.

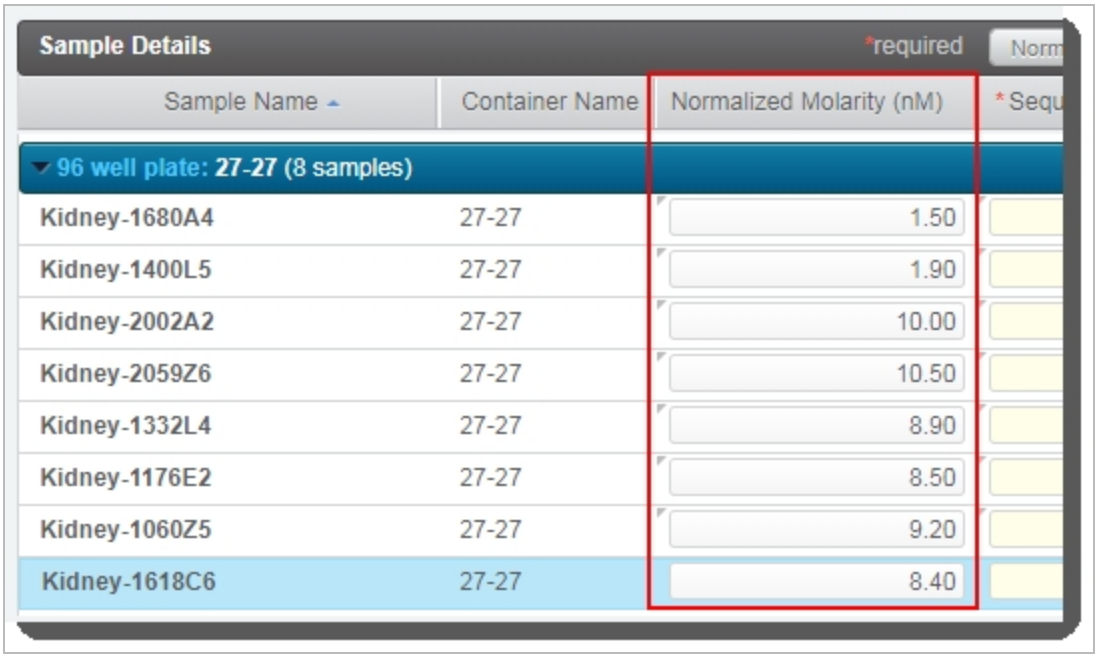
Step 1: Run Define Run Format (NovaSeq 6000 v3.0)

1. In Lab View, locate the Run Format (NovaSeq 6000 v3.0) protocol. You will see your samples queued for the Define Run Format (NovaSeq 6000 v3.0) step.



2. Add the samples to the Ice Bucket and click View Ice Bucket.
3. On the Ice Bucket screen, click Begin Work.
4. On the Record Details screen, in the Sample Details table, populate the following fields (values can vary across samples):
   - Loading Workflow Type - Select NovaSeq Standard or NovaSeq Xp from the drop-down list.

- Flowcell Type - Select SP, S1, S2, or S4.
- Final Loading Concentration (pM) - You may select from the two preset options - 225 (for PCR-free workflows) or 400 (for Nano workflows), or you may enter a different value.



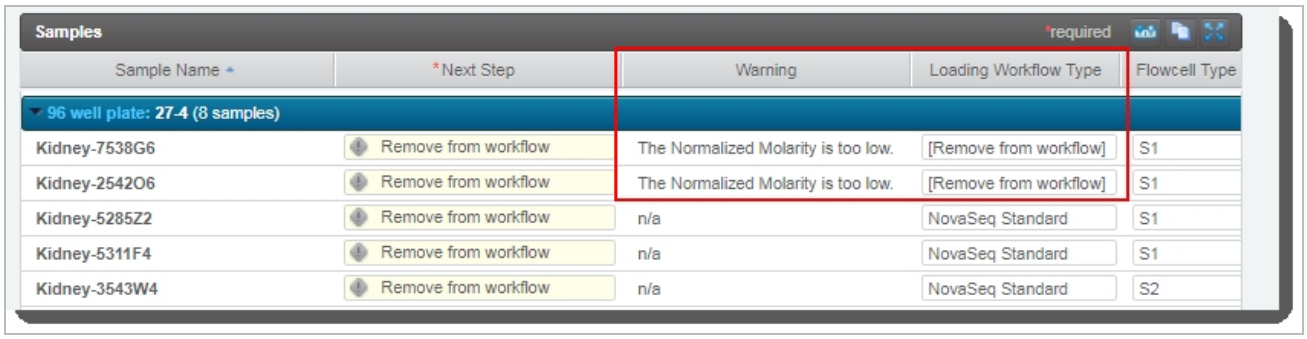
- The Normalized Molarity (nM) values are copied over from the previous step. If you did not populate this column during library prep, you can enter the values here.

5. Click Next Steps. This triggers the Next Steps automation, which:
   - Sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value, and requires it in order to successfully advance samples to the next step.
   - Calculates the Minimum Molarity.
   - Checks Normalized Molarity value. For samples with no Normalized Molarity value (i.e., empty value, not including 0), generates an error message informing the user that the field cannot be empty.
   - Compares each sample's Normalized Molarity value with the Minimum Molarity value.
6. On the Assign Next Steps screen:
   - In the Sample Details table, the Next Step for all samples is pre-populated with Remove from workflow - regardless of the Loading Workflow Type.

Do not change this value! If Next Step is not set to Remove from workflow, the routing script will not be able to route samples correctly.

   - For samples whose Normalized Molarity value was found to be lower than the Minimum Molarity value, the Loading Workflow Type is set to Remove from workflow and a message is recorded in the Warning field for the sample.

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At this point you have two options:

- Return to the Record Details screen and adjust the Normalized Molarity value so that it equals or exceeds the Minimum Molarity value. You will also need to set the Loading Workflow Type to NovaSeq Standard or NovaSeq Xp, as applicable.
- Complete the protocol without correcting the Normalized Molarity value. In this case, the samples in question will be removed from the LIMS workflow.

7. Click Finish Step. The Routing Script automation is triggered:
    - Samples whose Loading Workflow Type is set to Remove from workflow (i.e., where Normalized Molarity value is lower than the Minimum Molarity) are removed from the LIMS workflow.
    - Samples whose Loading Workflow Type is NovaSeq Standard are routed to the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.0) step. This is the first of two steps in *Protocol 2: NovaSeq Standard (NovaSeq 6000 v3.0)* on page 885.
    - Samples whose Loading Workflow Type is set to NovaSeq Xp are routed to the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.0) step. This is the first of three steps in *Protocol 3: NovaSeq Xp (NovaSeq 6000 v3.0)* on page 894.

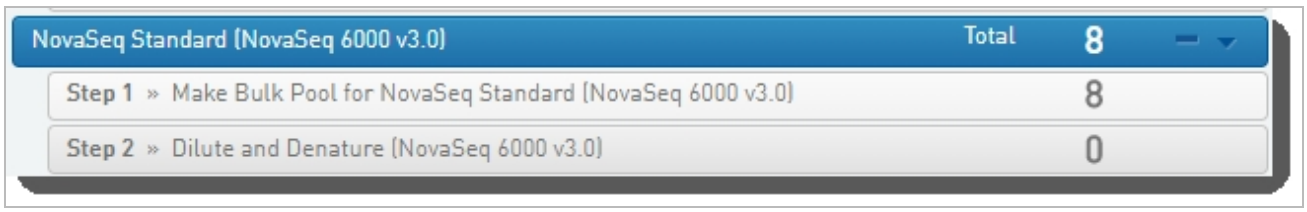
Protocol 2: NovaSeq Standard (NovaSeq 6000 v3.0)

In this protocol, samples are pooled and added to the library tube in preparation for the NovaSeq run. The protocol contains two steps:

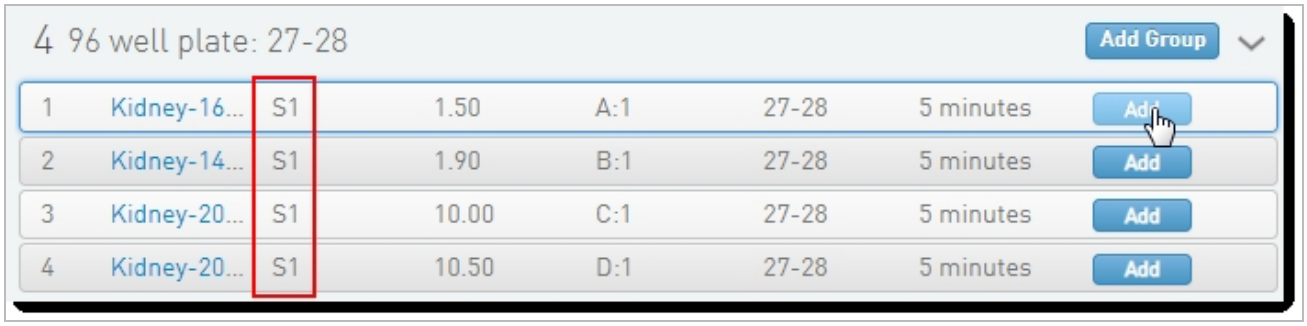
1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.0)
2. Dilute and Denature (NovaSeq 6000 v3.0)

Step 1: Run Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.0)

1. In Lab View, locate the NovaSeq Standard (NovaSeq 6000 v3.0) protocol. You will see your samples queued for the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v3.0) step.

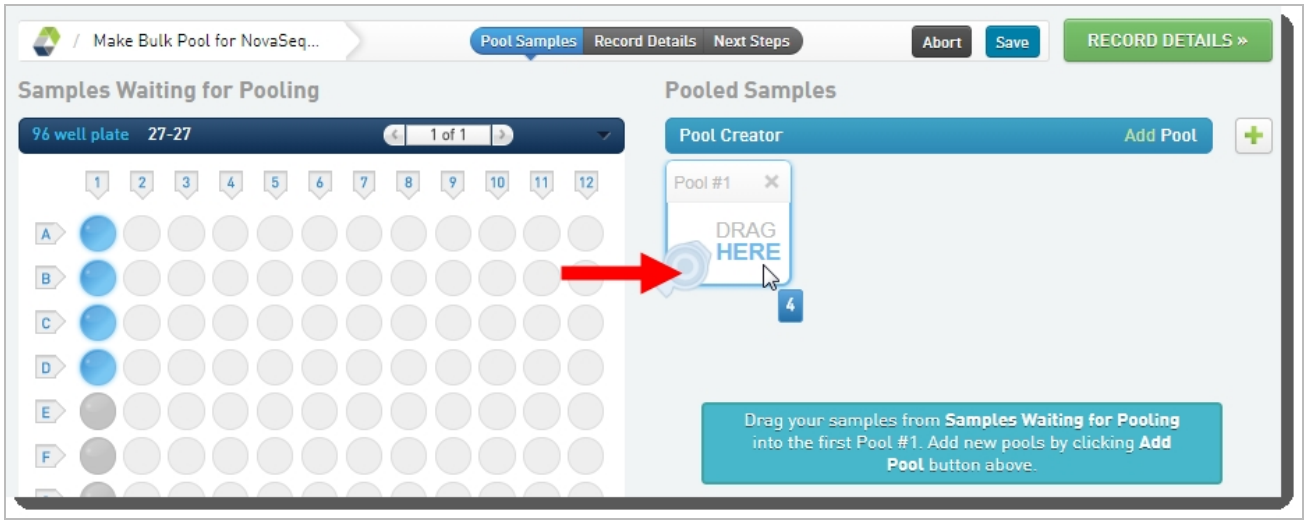


2. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucket and click View Ice Bucket.

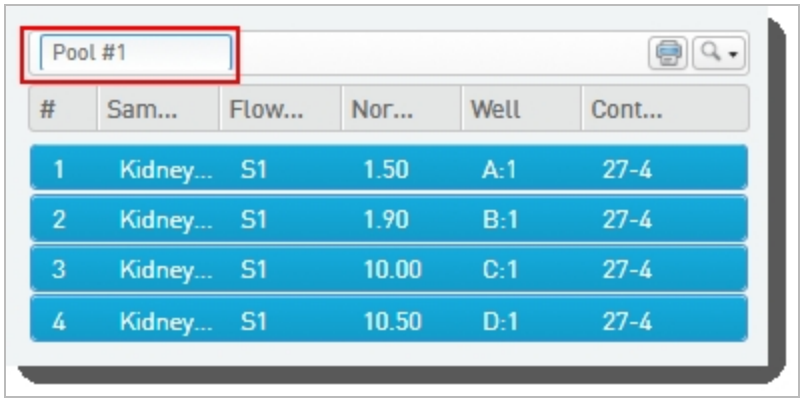


3. On the Ice Bucket screen, click Begin Work.
4. On the Pooling screen:
    - Create a pool by dragging samples into the Pool Creator.

You must only create one pool.

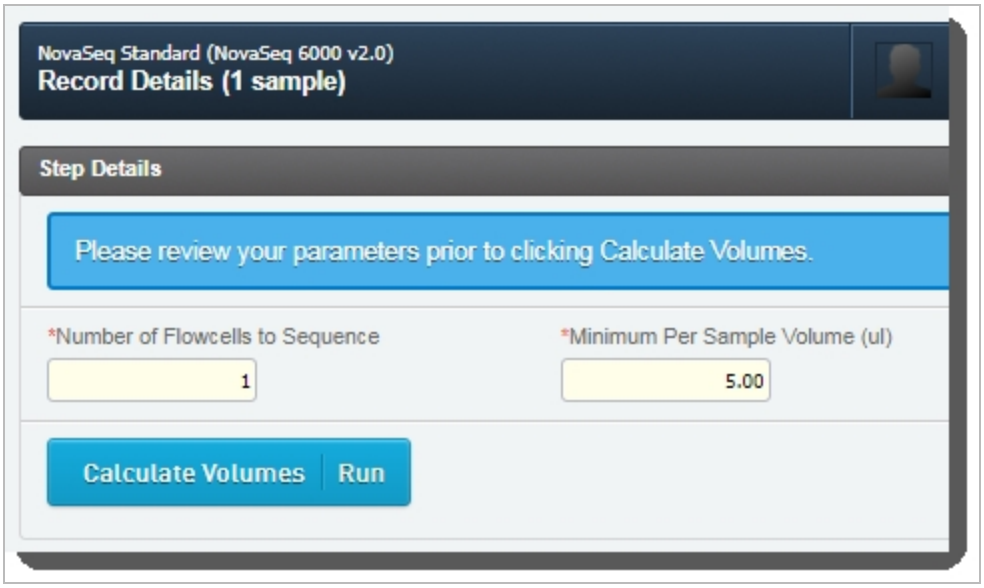


    - You can type a name for the pool, or accept the default name - Pool #1.



- Click Record Details.

5. On exit from the Pooling screen, the Validate Inputs Flowcell Type and Single Pool is triggered. The automation checks that:
   - All samples in the pool have been assigned the same Flowcell Type.
   - Only one pool has been created.
6. On the Record Details screen, the Step Details area contains two required fields:
   - Number of Flowcells to Sequence - The value entered in this field is used in volume calculations, to ensure that volumes are sufficient for the number of times the pool will be sequenced.
   - Minimum Per Sample Volume (ul) - The value in this field is used to calculate how much of each sample will be included in the pool. The field is pre-populated with the configured default value - 5 ul, but may be edited if required.

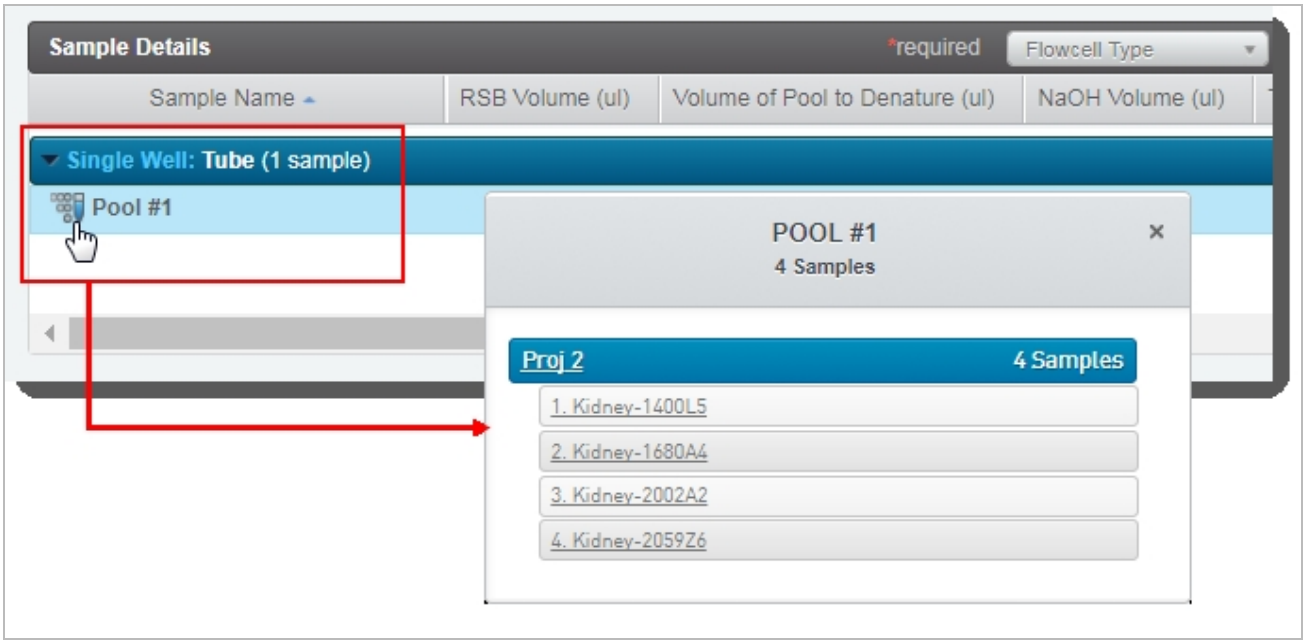


Assuming the default Minimum Per Sample Volume (ul) value of 5, for a given batch:

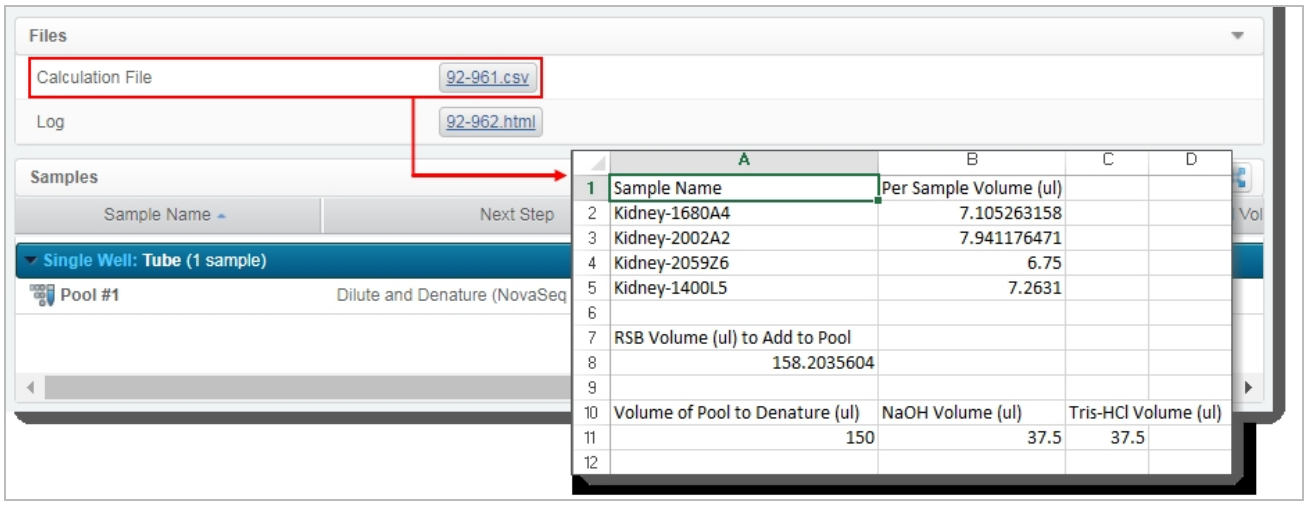
a. If the smallest Per Sample Volume (ul) value is less than 5, the LIMS automatically assigns a value of 5 to the sample's Adjusted Per Sample Volume (ul) field.
b. The LIMS then adjusts the Adjusted Per Sample Volume (ul) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

- In the Sample Details table, you can click on the pool icon to view details on the pool composition.

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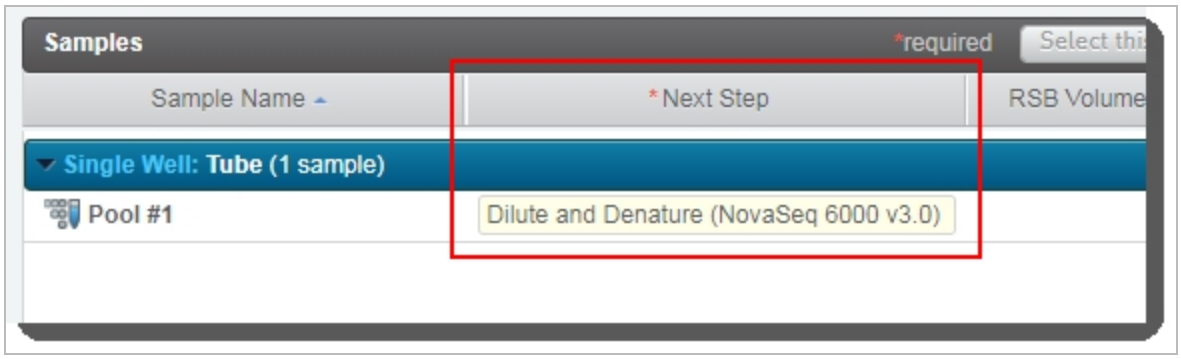


- Click Calculate Volumes. This triggers the Calculate Volumes automation, which performs volume calculations based on the selected Flowcell Type:
  - Calculates Bulk Pool Volume (ul) value.
  - Sets the value of the Bulk Pool Volume (ul) field, based on the selected Flowcell Type.
  - Calculates the Per Sample Volume (ul) value to be added to the pool.
  - Calculates the Total Sample Volume (ul) value.
  - If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (ul) value.
  - Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
  - Populates the Volume of Pool to Denature (ul), NaOH Volume (ul) and Tris-HCl Volume (ul) columns of the Sample Details table. Values are set by a script and are not editable by the user.
  - The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains volume information about the pool and the individual samples it contains. Click on the file to download it and then open it in Excel.



- Click Next Steps. This triggers the Set Next Step automation. This automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol - Dilute and Denature (NovaSeq 6000 v3.0).

7. On the Assign Next Steps screen, the next step for samples is already set to the next step in the workflow: Dilute and Denature (NovaSeq 6000 v3.0).

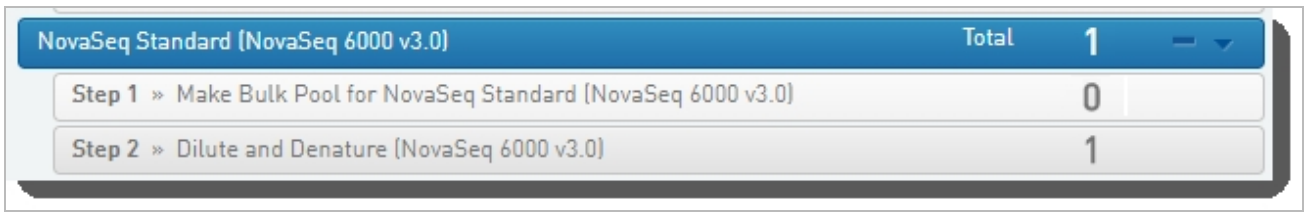


8. Click Finish Step.

At the end of this step, the pool of samples automatically advances to the Dilute and Denature (NovaSeq 6000 v3.0) step.

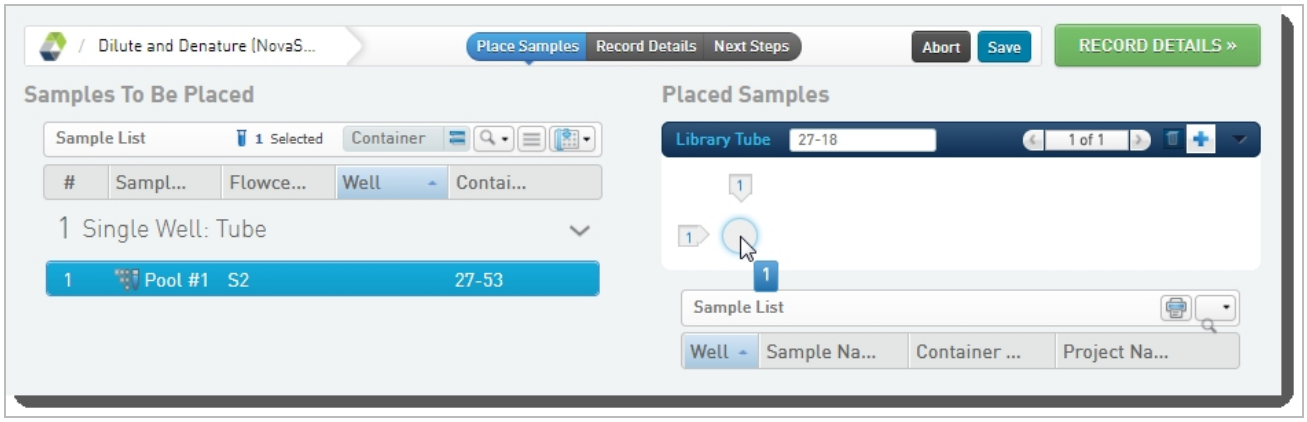
Step 2: Run Dilute and Denature (NovaSeq 6000 v3.0)

1. In Lab View, locate the NovaSeq Standard (NovaSeq 6000 v3.0) protocol. You will see the pool of samples queued for the Dilute and Denature (NovaSeq 6000 v3.0) step.

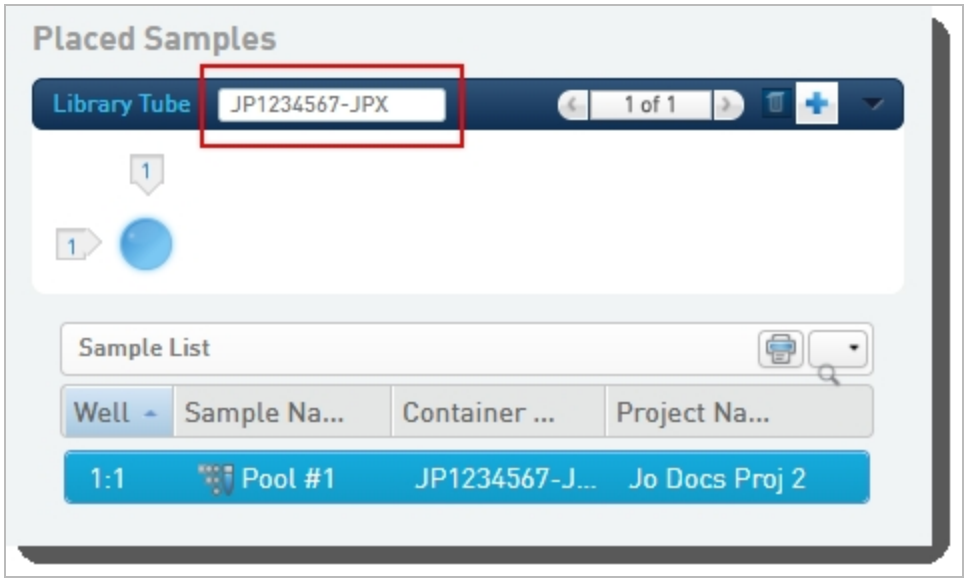


2. Add the samples to the Ice Bucket and click View Ice Bucket.

3. On the Ice Bucket screen, click Begin Work. The Validate Single Input automation is triggered. This automation checks that there is only one container input to the step.
4. On the Placement screen:
    - Drag the pool into the library tube in the Placed Samples area.



    - Scan or type the barcode of the library tube into the Library Tubefield.



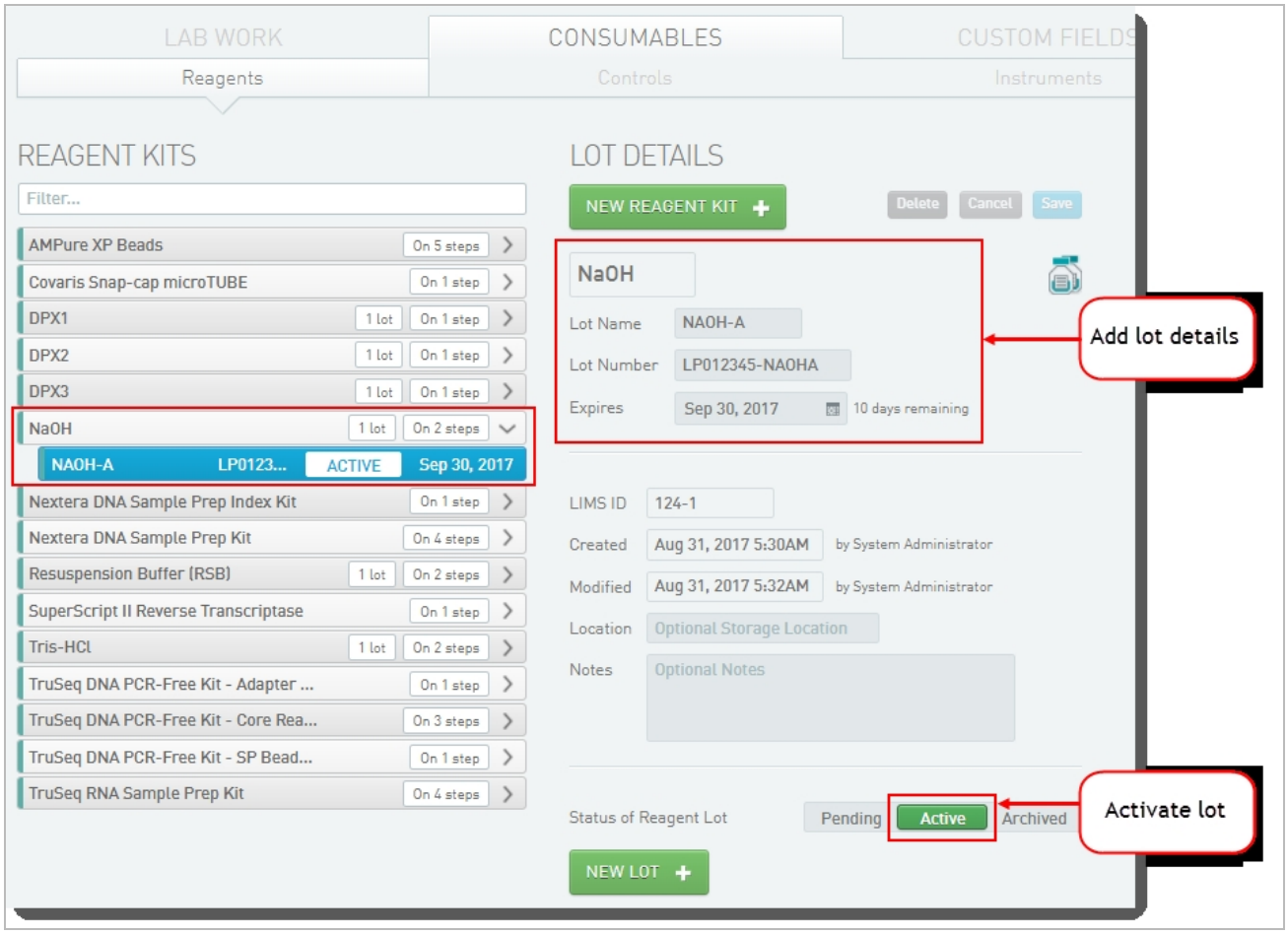
    - Click Record Details.

    On exit of the Placement screen, the Validate Library Tube Barcode automation checks that the library tube barcode conforms to the barcode mask [A-Za-z]{2}[0-9]{7}-[A-Za-z]{3}, and displays an error message if this is not the case. This automation also copies the Flowcell Type and Loading Workflow Type values from step inputs to outputs.
5. On the Record Details screen, the Reagent Lot Tracking section lets you track the NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. You will need to add and activate lots for these reagents.

    To add and activate reagent lots:

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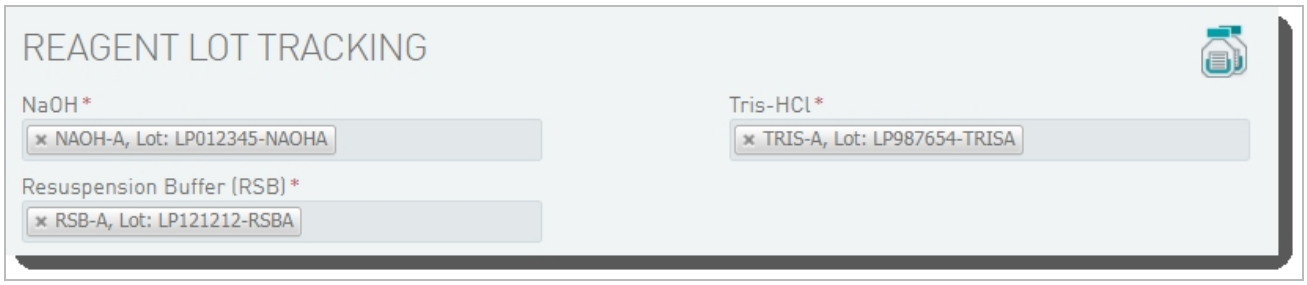
- In a new browser tab, open an additional instance of the LIMS. LIMS v5.0:
  - Navigate to the Consumables configuration screen.
  - LIMS v4.2: Navigate to the Reagents and Controls screen.
- Select the NaOH reagent kit and click New Lot.
- Enter the lot details and click Save.
- Set the Status of Reagent Lot toggle switch to Active.



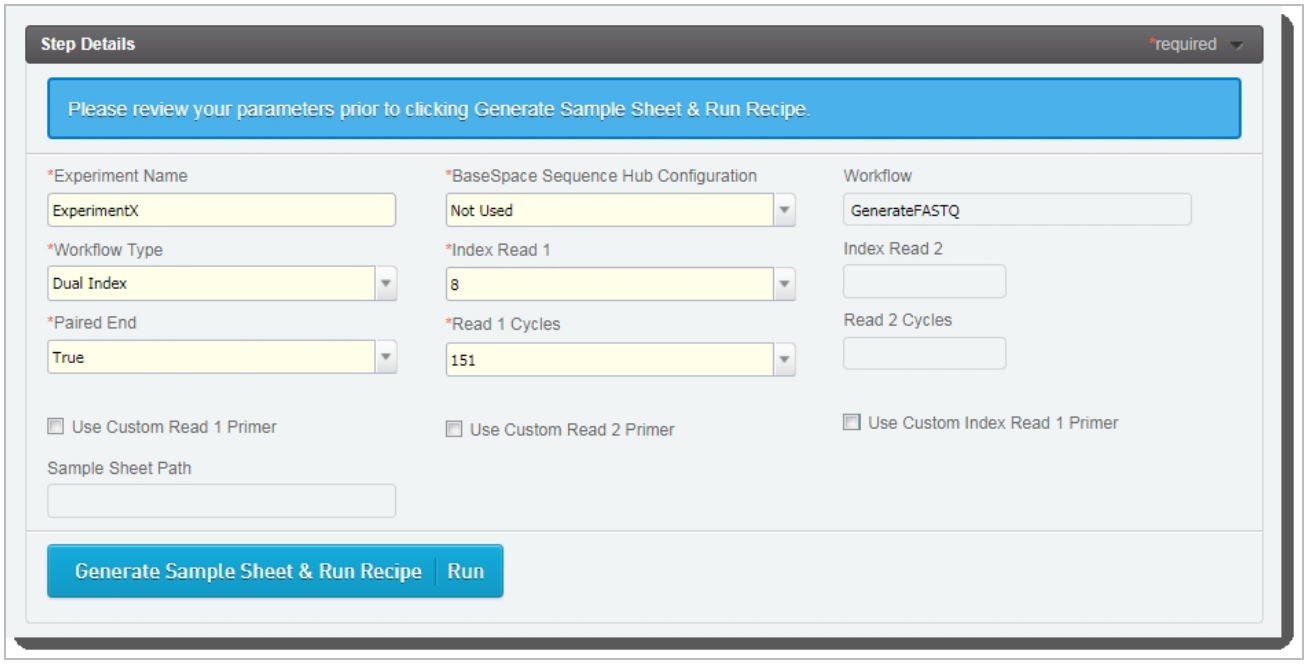
- Repeat this process to add and activate Resuspension Buffer and Tris-HCl reagent lots.
- Return to your original working browser tab and refresh the page.

6. In the Reagent Lot Tracking section, select from the active lots displayed in each drop-down list.

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7. The fields displayed on the Record Details screen are used to set up the run and generate the sample sheet.



Some of these fields are auto-populated and some must be completed by the user (see the following table for details):

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. No spaces.
BaseSpace Sequence Hub Configuration	Select from preset options: Not Used, Run Monitoring Only, or Run Monitoring and Storage
Workflow	Automatically populated with preset - GenerateFASTQ
Workflow Type	Select from preset options: No Index, Single Index, Dual Index, or Custom
Index Read 1	Select from preset options: 0, 6, or 8 - or type a value between 0 and 20.
Index Read 2	Select from preset options: 0, 6, or 8 - or type a value between 0 and 20.

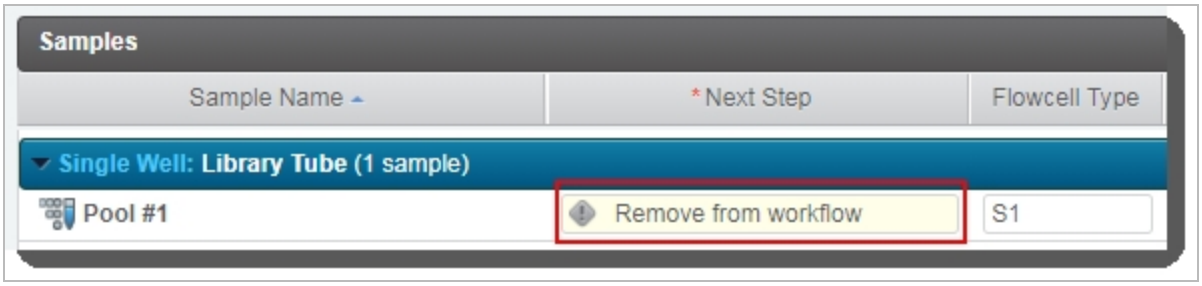
Field	Value
Paired End	Select from preset options: True or False
Read 1 Cycles	Select from preset options: 251, 151, 101, or 51 - or type a value between 1 and 251. *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Read 2 Cycles	Select from preset options: 251, 151, 101, or 51 - or type a value between 1 and 251. *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Use Custom Read 1 Primer	Select if applicable. LIMS v5.0.5 and later: See *Configuration Update for NovaSeq Integration with Clarity LIMS v5.0.5 and Later* on page 1237 documentation.
Use Custom Read 2 Primer	Select if applicable. LIMS v5.0.5 and later: See *Configuration Update for NovaSeq Integration with Clarity LIMS v5.0.5 and Later* on page 1237 documentation.
Use Custom Index Read 1 Primer	Select if applicable. LIMS v5.0.5 and later: See *Configuration Update for NovaSeq Integration with Clarity LIMS v5.0.5 and Later* on page 1237 documentation.
Output Folder	Enter network path for sequencing run folder. For example: \\networkshare\run_data
Use Custom Recipe	Select if applicable.
Custom Recipe Path	If you selected the Use Custom Recipe option, enter the path to the custom recipe file to be used.

8. On the Record Details screen, click Validate Run Setup and Generate Sample Sheet . This triggers the automation script, which:
    - Validates the parameters entered on the Record Details screen.
    - Generates the sample sheet and attaches it to the placeholder in the Files area of the Record Detailsscreen.



- Click Next Steps.

9. On the Assign Next Steps screen, the Next Step for samples is pre-populated with Remove from workflow. The Routing Script automation expects this value, and requires it in order to successfully advance samples to the next step.



Do not change this value. If Next Step is not set to Remove from workflow, the routing script will not be able to route samples correctly.

10. Click Finish Step.
    On exit from the step:
    - The Routing Script automation is triggered and samples are routed to Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0).
    - In Lab View, the pool of samples is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0) step.

At this point in the workflow, the user interaction ends. Proceed to *Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0)* on page 908

Do not add samples to the Ice Bucket or start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0) step. The integration will do this automatically.

Protocol 3: NovaSeq Xp (NovaSeq 6000 v3.0)

In this protocol, samples are pooled and added to lanes on the NovaSeq flow cell. The flow cell type is determined by the option selected in the Define Run Format (NovaSeq 6000 v3.0) step.

The protocol contains three steps:

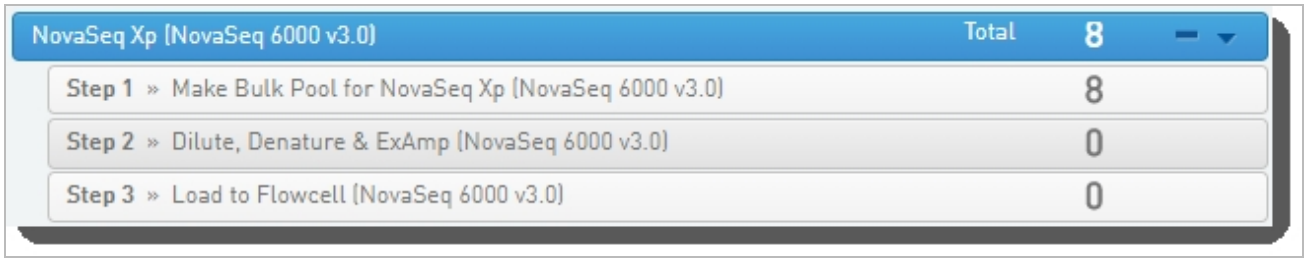
1. Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.0)

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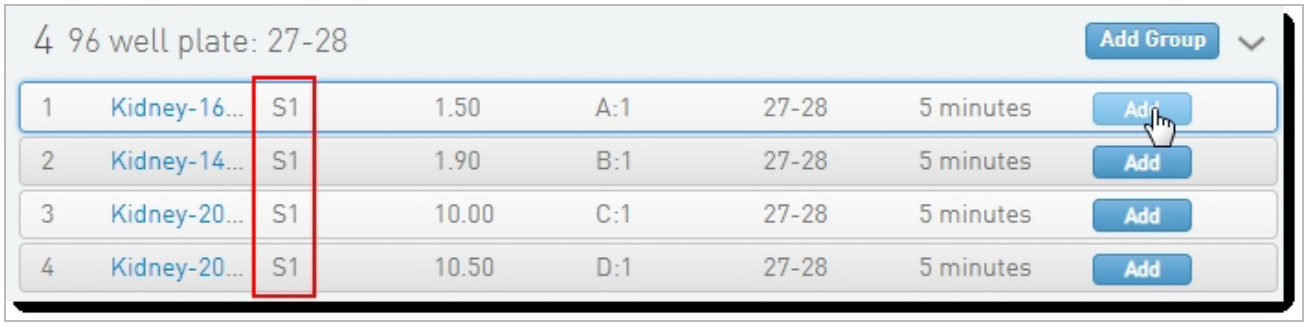
2. Dilute, Denature and ExAmp (NovaSeq 6000 v3.0)
3. Load to Flowcell (NovaSeq 6000 v3.0)

Step 1: Run Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v3.0)

1. In Lab View, locate the NovaSeq Standard (NovaSeq 6000 v3.0) protocol. You will see your samples queued for the Make Bulk Pool for NovaSeq XP (NovaSeq 6000 v3.0) step.

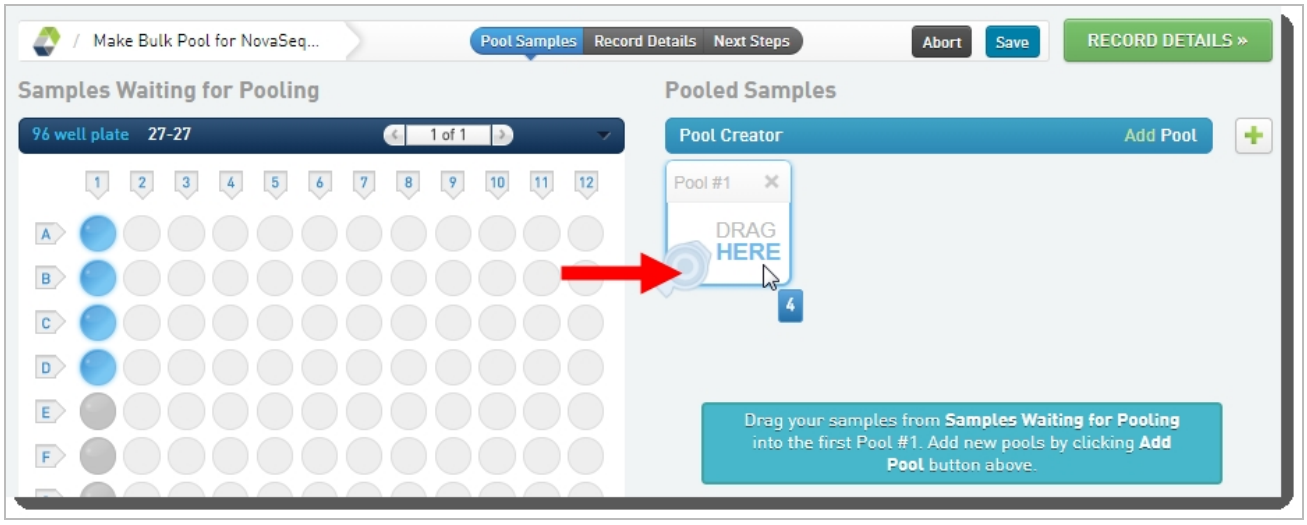


2. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucket and click View Ice Bucket.



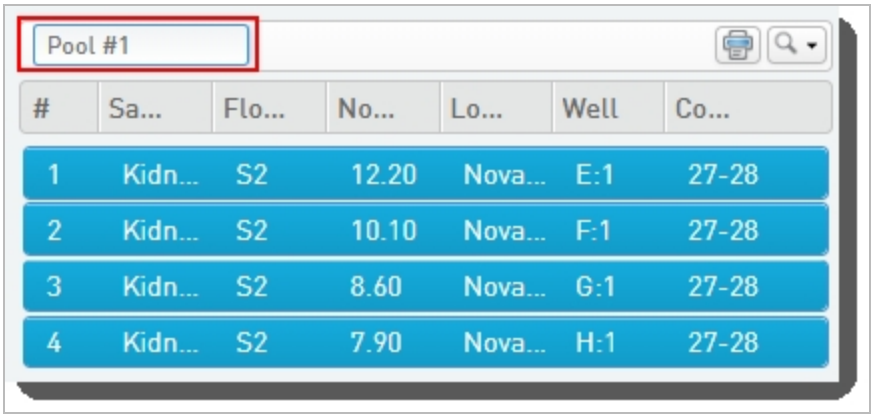
3. On the Ice Bucket screen, click Begin Work.
4. On the Pooling screen:
    - Create a pool by dragging samples into the Pool Creator.

    You must only create one pool.



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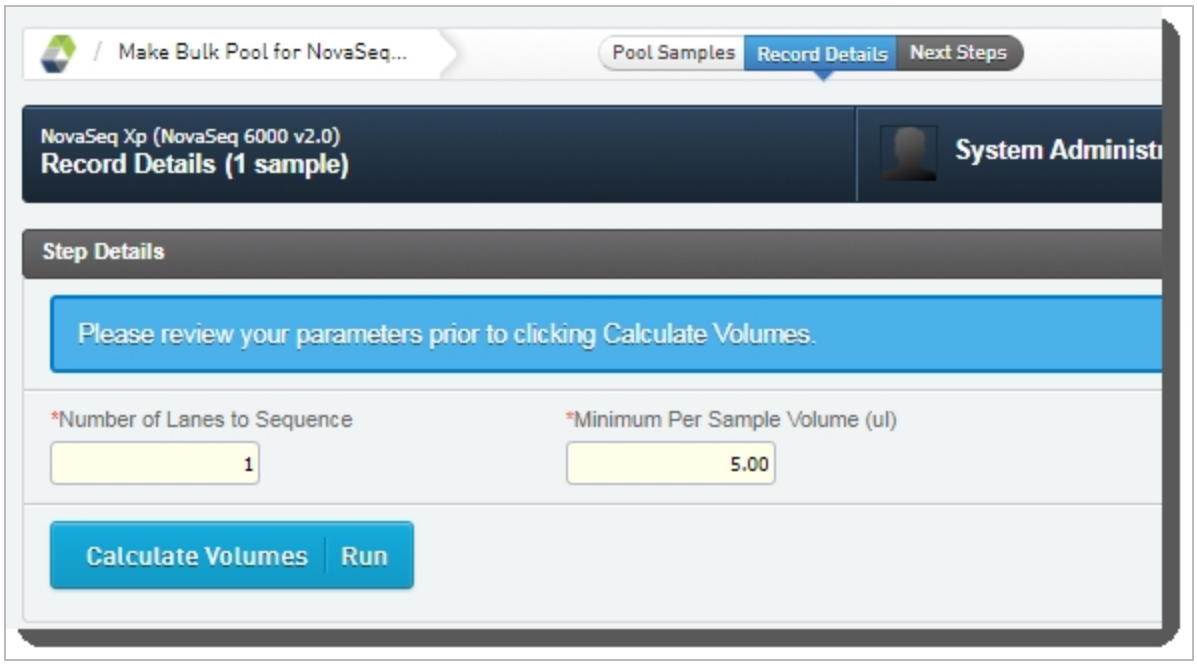
- You can type a name for the pool, or accept the default name - Pool #1.



- Click Record Details.

5. On exit from the Pooling screen, the Validate Inputs Flowcell Type and Single Pool is triggered. The automation checks that:
    - All samples in the pool have been assigned the same Flowcell Type.
    - Only one pool has been created
6. On the Record Details screen, the Step Details area contains two required fields:
    - Number of Lanes to Sequence - The value entered in this field is used in volume calculations, to ensure that volumes are sufficient for the number of times the pool will be sequenced.
    - Minimum Per Sample Volume (ul) - The value in this field is used to calculate how much of each sample will be included in the pool. The field is pre-populated with the configured default value - 5 ul, but may be edited if required.

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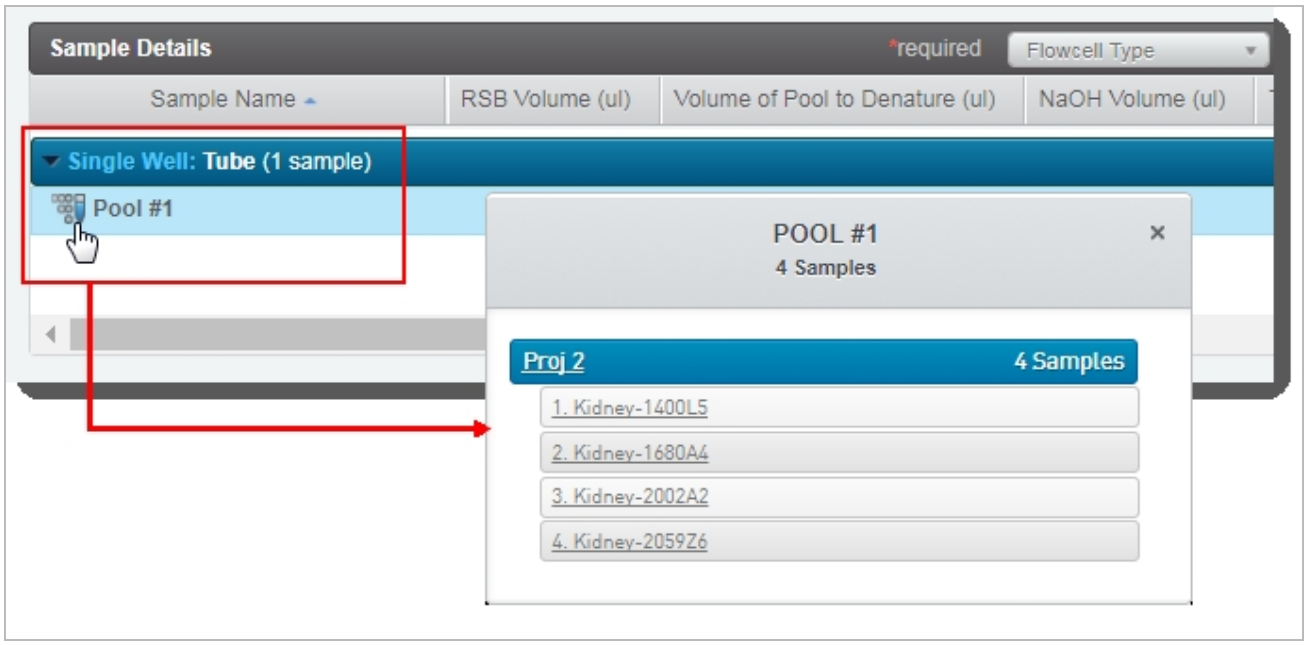


Assuming the default Minimum Per Sample Volume (ul) value of 5, for a given batch:

a. If the smallest Per Sample Volume (ul) value is less than 5, the LIMS automatically assigns a value of 5 to the sample's Adjusted Per Sample Volume (ul) field.

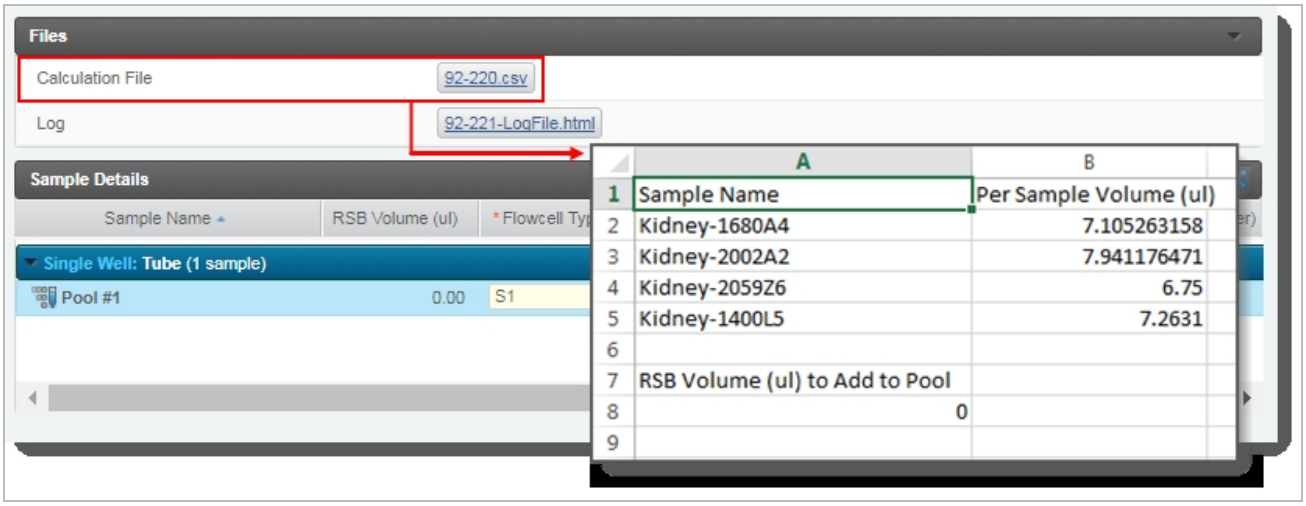
b. The LIMS then adjusts the Adjusted Per Sample Volume (ul) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

- In the Sample Details table, you can click on the pool icon to view details on the pool composition.



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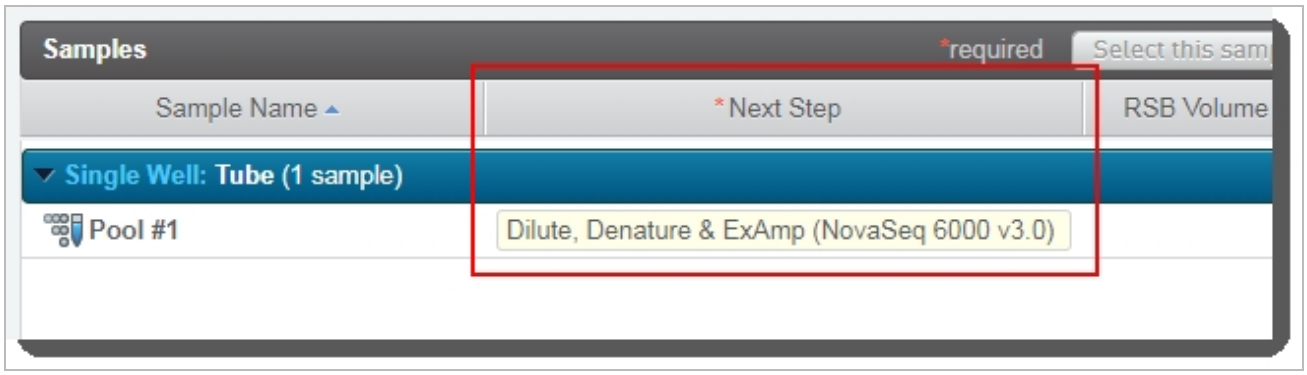
- Click Calculate Volumes. This triggers the Calculate Volumes automation, which performs volume calculations based on the selected Flowcell Type:
  - Calculates Bulk Pool Volume (ul) value.
  - Calculates the Per Sample Volume (ul) value to be added to the pool.
  - Calculates the Total Sample Volume (ul) value.
  - If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (ul) value.
  - The automation also populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
  - The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains volume information about the samples and RSB buffer to add to the pool. Click on the file to download it and then open it in Excel.



- Click Next Steps. This triggers the Set Next Step automation, which:
  - Copies the Flowcell Type values from the step inputs to the step outputs.
  - Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol - Dilute, Denature and ExAmp (NovaSeq 6000 v3.0).

7. On the Assign Next Steps screen, the next step for samples is already set to the next step in the workflow - Dilute, Denature and ExAmp (NovaSeq 6000 v3.0).

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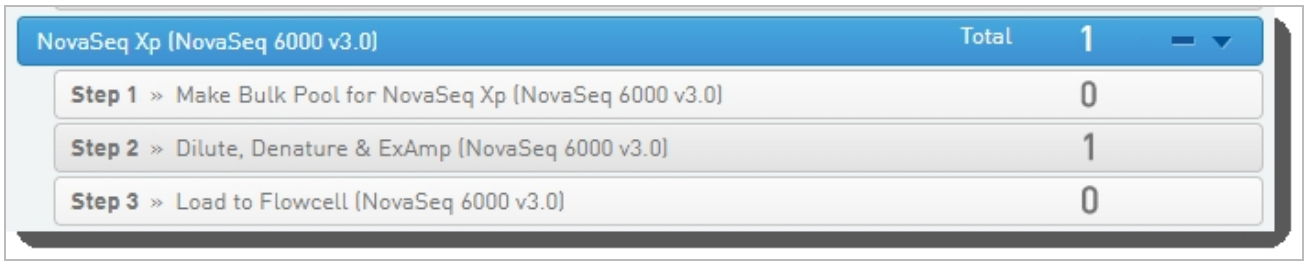


8. Click Finish Step.

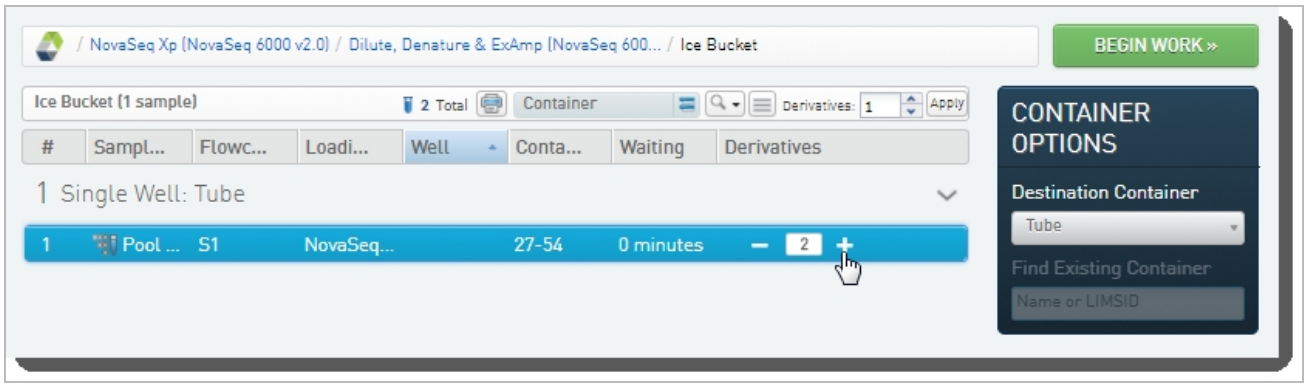
At the end of this step, the pool of samples automatically advances to the Dilute, Denature and ExAmp (NovaSeq 6000 v3.0) step.

Step 2: Run Dilute, Denature and ExAmp (NovaSeq 6000 v3.0)

1. In Lab View, locate the NovaSeq Xp (NovaSeq 6000 v3.0) protocol. You will see the pool of samples queued for the Dilute, Denature and ExAmp (NovaSeq 6000 v3.0) step.



2. Add the pool to the Ice Bucket and click View Ice Bucket.
3. [Optional] On the Ice Bucket screen, set the number of derivatives to create (these will be placed into the flow cell lanes) and click Begin Work.



4. On entry to the Record Details screen, the Calculate Volumes automation is triggered. This automation sets the following values based on the selected Flowcell Type:
    - BP Aliquot Volume (ul)
    - NaOH Volume (ul)

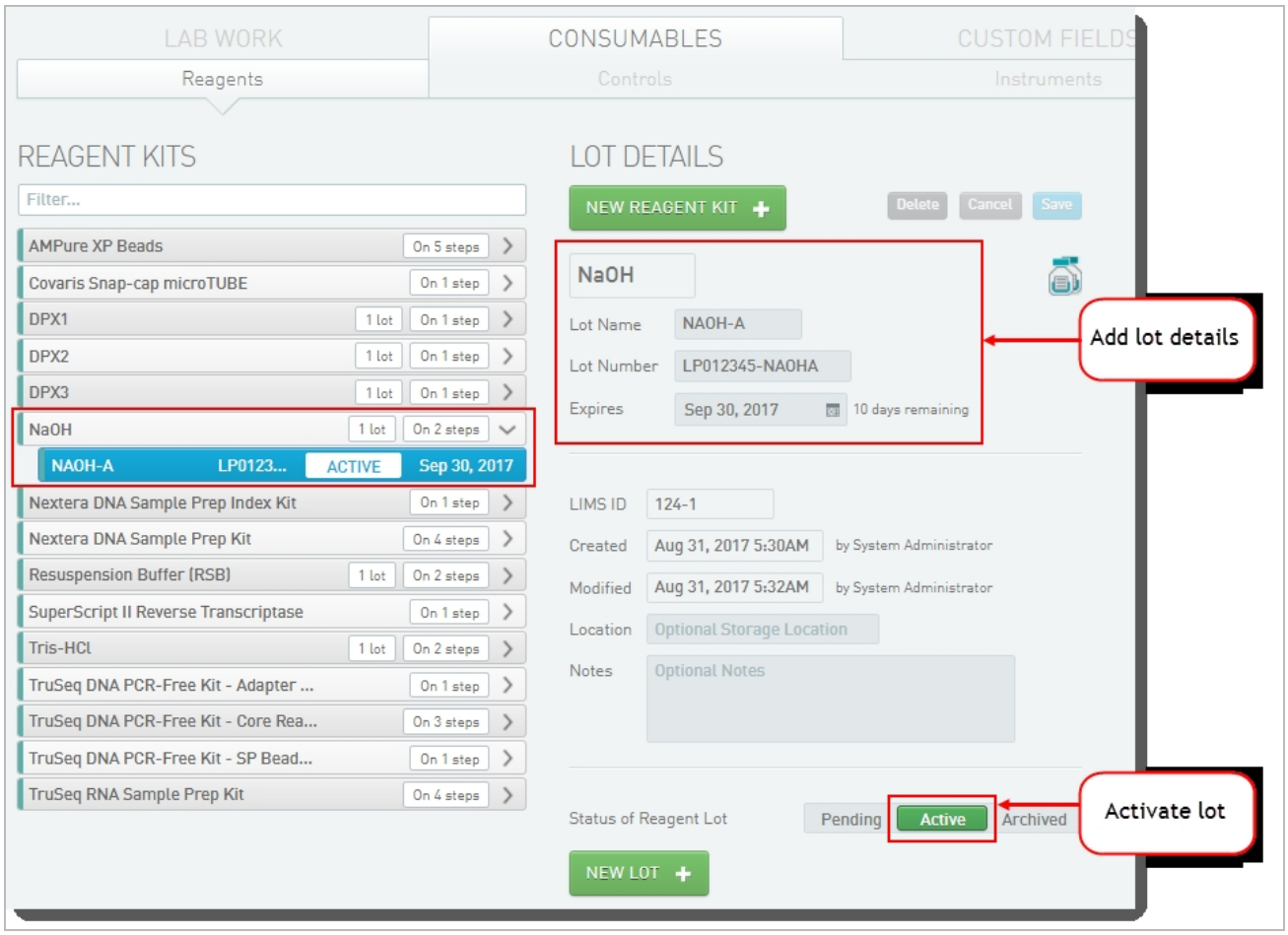
- Tris-HCl Volume (ul)
- DPX1 Volume (ul)
- DPX2 Volume (ul)
- DPX3 Volume (ul)
- Mastermix per Lane (ul)
- The automation also populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
- The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains information about the DPX Mastermix volume, and the volume of Mastermix, NaOH. and Tris-HCl to add per working pool (see the next step).

5. On the Record Details screen, the Reagent Lot Tracking section lets you track the DPX1, DPX2, DPX3, NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. You will need to add and activate lots for these reagents

   To add and activate reagent lots:

   - In a new browser tab, open an additional instance of the LIMS. LIMS v5.0:
   – Navigate to the Consumables configuration screen.LIMS v4.2:
   – Navigate to the Reagents and Controls screen.
   - Select one of the reagent kits listed above (NaOH shown below) and click New Lot.
   - Enter the lot details and click Save.
   - Set the Status of Reagent Lot toggle switch to Active.

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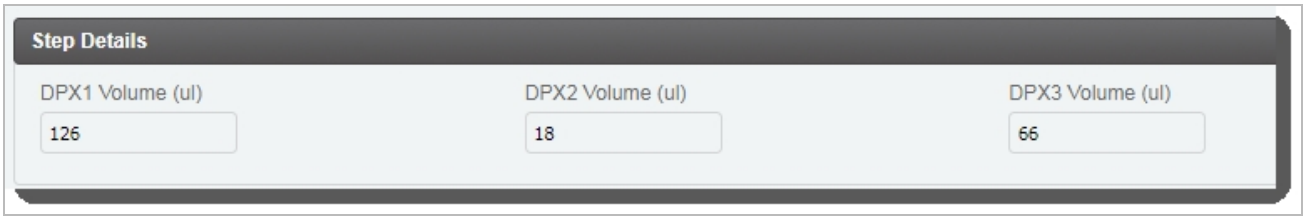
- Repeat this process to add and activate the remaining reagent lots.
- Return to your original working browser tab and refresh the page.

6. On the Record Details screen:
   - In the Reagent Lot Tracking section, select from the active lots displayed in each drop-down list.

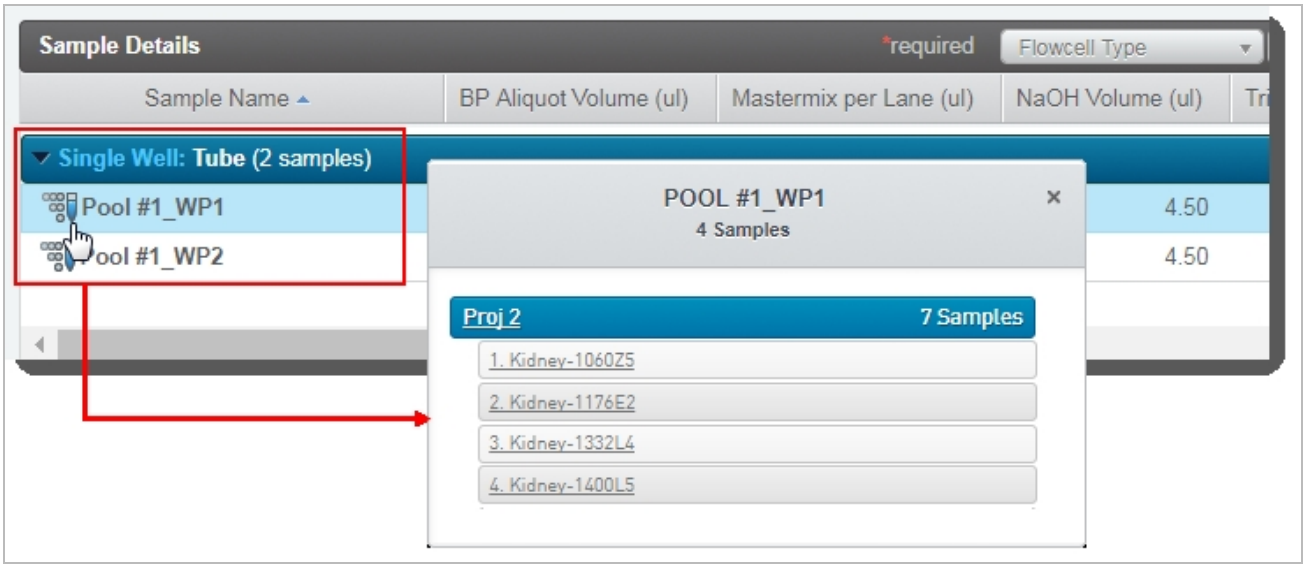


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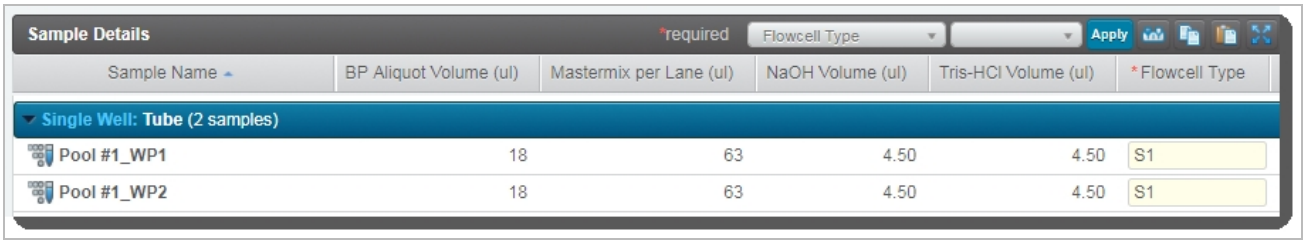
- In the Step Details area, the DPX1, DPX2, and DPX3 reagent volume values are already populated. These values are set by a script and are not editable by the user running the step.



- In the Sample Details table, you can click on the pool icon to view details on the working pool composition.

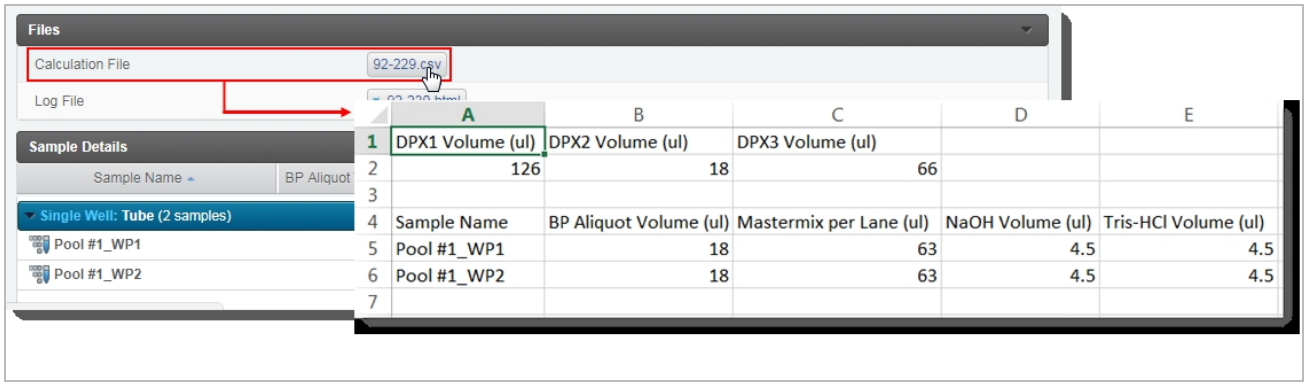


- The BP Aliquot, Mastermix per lane, NaOH, and Tris-HCl volume values for each working pool are already populated. These values are set by a script and are not editable by the user running the step.
- The Flowcell Type and Loading Workflow Type columns are already populated.
- The working pool number is appended to the bulk pool name. This allows you to quickly identify which working pools are derived from the same bulk pool.

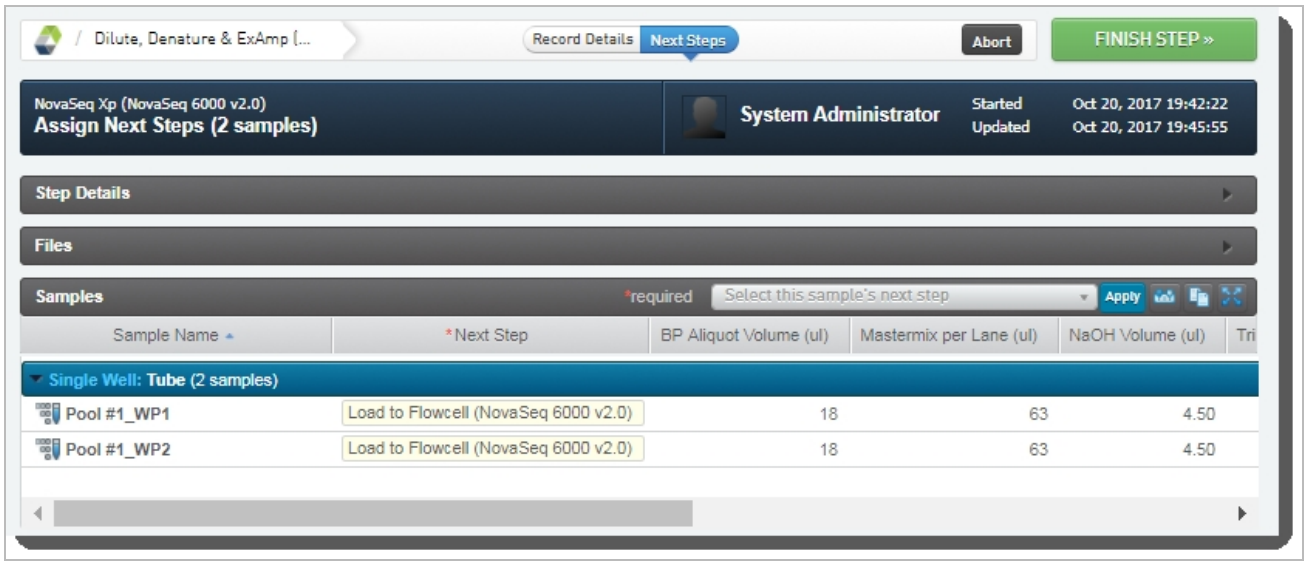


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7. In the Files area, click on the Calculation File (CSV) to open it and view details on the DPX Mastermix volume, and the volume of Mastermix, NaOH. and Tris-HCl to add per working pool.



8. Click Next Steps.
9. On the Assign Next Steps screen, the next step is already set to Load to Flowcell (NovaSeq 6000 v3.0).



10. Click Finish Step.

Step 3: Run Load to Flowcell (NovaSeq 6000 v3.0)

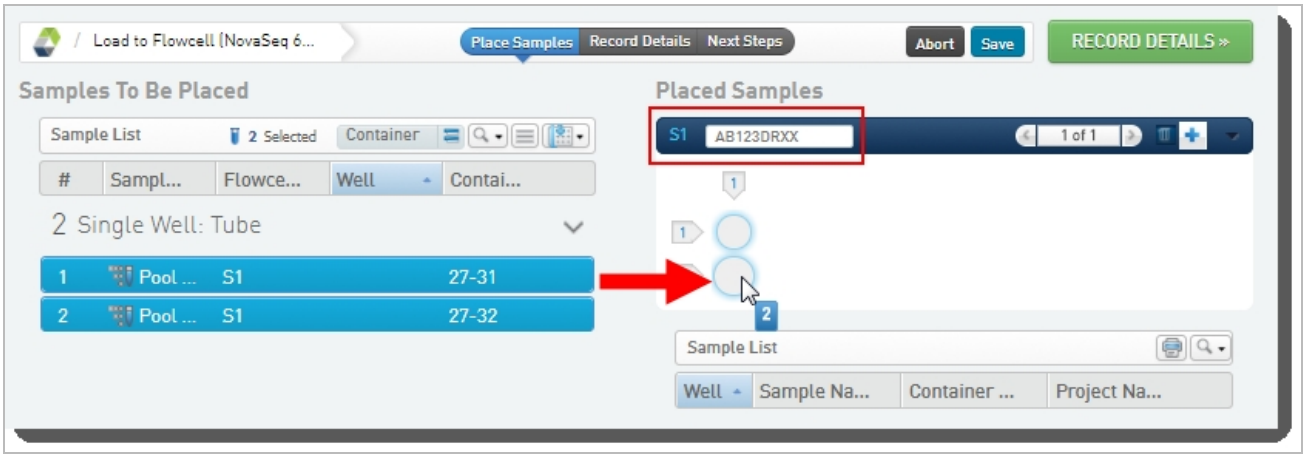
1. On the Ice Bucket screen:
    - In the Container Options panel, select the appropriate flow cell type from the Destination Containerdrop-down list.

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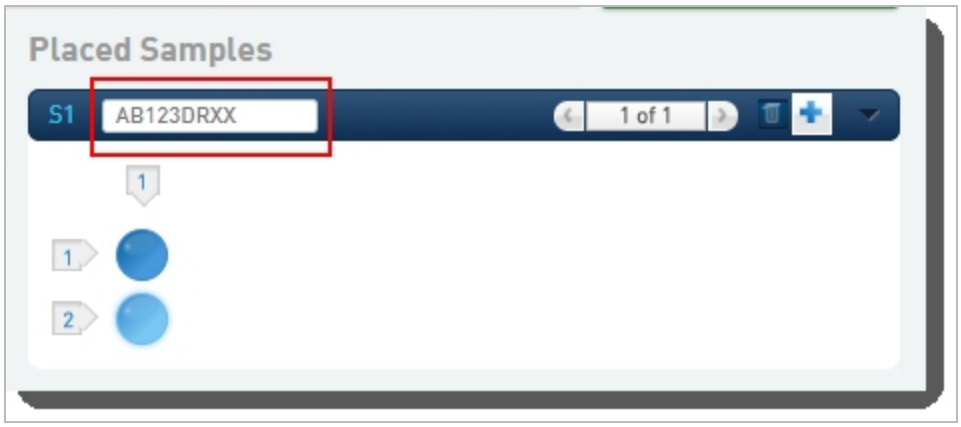
- Click Begin Work.

2. The Validate Inputs and Selected Container automation checks that:
   - The Flowcell Type field has been set to a valid value (SP, S1, S2, or S4), and that all inputs have the same value for this field.
   - The container type selected matches the value in the Flowcell Type field.
   - The number of outputs matches the number of lanes on the selected flow cell type. If validation fails, an error message informs the user that the number of working pools does not match the number of lanes available on the flow cell.
3. On the Placement screen:
   - Drag the pool(s) from the left of the screen over into the Placed Samples area on the right.



- Scan or type the barcode of the flow cell into the Flow Cell field.

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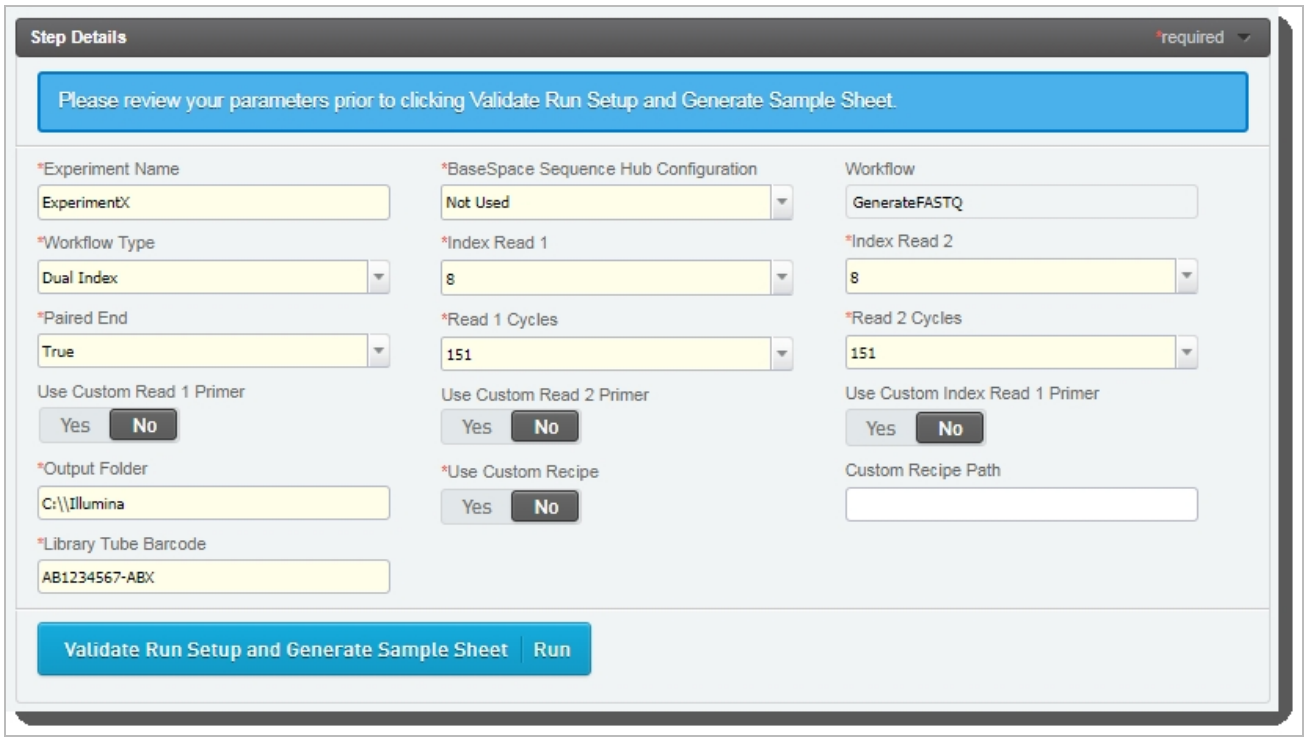


- Click Record Details.

  On exit of the Placement screen, the Validate Flowcell Barcode automation checks that the container barcode conforms to the barcode mask for the selected Flowcell Type, as listed in the following table. This automation also copies the Flowcell Type and Loading Workflow Type field values from step inputs to outputs.

Flow Cell Type	Barcode Mask
SP	[A-Za-z0-9]{5}DRXX
S1	[A-Za-z0-9]{5}DRXX
S2	[A-Za-z0-9]{5}DMXX
S4	[A-Za-z0-9]{5}DSXX

4. The fields displayed on the Record Details screen are used to set up the run and generate the sample sheet.



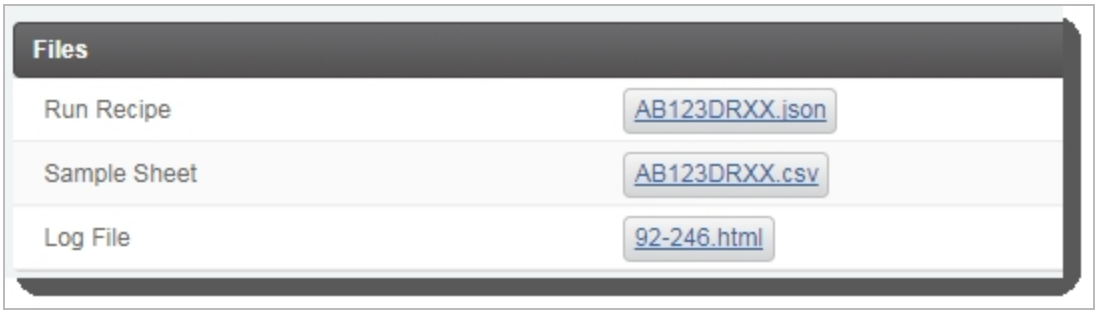
Some of these fields are auto-populated and some must be completed by the user (see the following table for details).

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. No spaces.
BaseSpace Sequence Hub Configuration	Select from preset options: Not Used, Run Monitoring Only, or Run Monitoring and Storage
Workflow	Automatically populated with preset - GenerateFASTQ.
Workflow Type	Select from preset options: No Index, Single Index, Dual Index, or Custom
Index Read 1	Select from preset options: 0, 6, or 8 - or type a value between 0 and 20.
Index Read 2	Select from preset options: 0, 6, or 8 - or type a value between 0 and 20.
Paired End	Select from preset options: True or False
Read 1 Cycles	Select from preset options: 251, 151, 101, or 51 - or type a value between 1 and 251. *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.

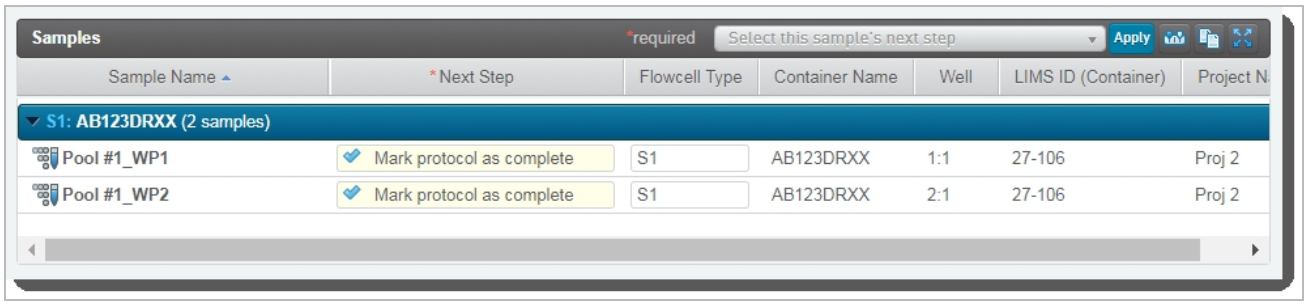
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Field	Value
Read 2 Cycles	Select from preset options: 251, 151, 101, or 51 - or type a value between 1 and 251. *Value of 251 is only supported for SP flow cell type. For all other flow cell types, maximum value is 151.
Use Custom Read 1 Primer	Select if applicable. LIMS v5.0.5 and later: See *Configuration Update for NovaSeq Integration with Clarity LIMS v5.0.5 and Later* on page 1237.
Use Custom Read 2 Primer	Select if applicable. LIMS v5.0.5 and later: See *Configuration Update for NovaSeq Integration with Clarity LIMS v5.0.5 and Later* on page 1237.
Use Custom Index Read 1 Primer	Select if applicable. LIMS v5.0.5 and later: See *Configuration Update for NovaSeq Integration with Clarity LIMS v5.0.5 and Later* on page 1237.
Output Folder	Enter network path for sequencing run folder. For example: \\networkshare\run_data
Use Custom Recipe	Select if applicable.
Custom Recipe Path	If you selected the Use Custom Recipe option, enter the path to the custom recipe file to be used.
Library Tube Barcode	Scan the library tube barcode.

5. Click Validate Run Setup and Generate Sample Sheet.
    - This triggers the automation script, which: Validates the parameters entered on the Record Details screen.
    - Generates the sample sheet and attaches it to the placeholder in the Files area of the Record Details screen.



    - Click Next Steps.
6. On the Assign Next Steps screen, the Next Step field for samples is pre-populated with Mark protocol as complete.



7. Click Finish Step.

At this point in the workflow, the user interaction ends. The flow cell is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0) step.

Proceed to *Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0)* on page 908.

Do not add samples to the Ice Bucket or start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0) step. The integration will do this automatically.

Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0)

This protocol contains a single fully-automated step - AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0).

The integration starts the step automatically and data from the run is parsed back into the LIMS. No user interaction is required. However, the user may open and review the various stages of the step in the LIMS (see *Review Run Data* on page 908 section below).

By default, the Sequencer API is used to set the next step for output samples.

The PCWP includes a Set Next Steps automation configured on the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0) step. You can use this automation to override the default next step behavior. See *NovaSeq 6000 Integration v3.2.1 Configuration and User Interaction* on page 798 for details.

Review Run Data

Read summary metrics are recorded for the library pool. Once the run is complete, you can open the step and review these metrics on the Next Steps screen - in the Step Data section and the Sample Details table.

Step Data Section

You should see values populated in the following step UDFs/master step fields:

- Run ID
- Run Status
- Output Folder
- Current Cycle

- Current Read
- Loading Workflow Type
- Flow Cell ID
- Flow Cell Side
- Flow Cell Mode
- Flow Cell Part Number*
- Flow Cell Lot Number
- Flow Cell Expiration Date
- Instrument ID
- Instrument Type
- Instrument Control Software Version
- Firmware Version
- RTA Version
- Sequencing Log

** Part number of all consumables is not available in the payload sent from the NVCS to the LIMS. This issue will be fixed in the upcoming NVCS release.*

Sample Details Table

You should see summary metrics populated in the UDFs/global fields listed below. Values are aggregated across all lanes. Some values - for example Yield PF (Gb) R1 - are summed, while others are averaged.:

- Yield PF (Gb) R1
- Yield PF (Gb) R2*
- Reads PF (M) R1*
- Reads PF (M) R2*
- Cluster Density (K/mm^2) R1
- Cluster Density (K/mm^2) R2
- %PF R1
- %PF R2
- % Bases >=Q30 R1
- % Bases >=Q30 R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2

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- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- % Aligned R1
- % Aligned R2
- % Error Rate R1
- % Error Rate R2

** For R2 Yield, R2 Error rate, and R2 Q30, there are discrepancies between the values shown in the Illumina SAV software and in the payload sent from the NVCS to the LIMS. This issue will be fixed in the upcoming NVCS release.*

How the Integration Works

The following steps summarize how the Sequencer API integration works. For more detailed information, see the *NovaSeq 6000 Integration v3.2.1 Configuration and User Interaction* on page 798.

1. When the user begins setting up a run on the NovaSeq instrument, NVCS sends a request for the run recipe.
   a. The Sequencer API validates that sample(s) and container(s) are correctly queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0) step.
   b. The LIMS sends a JSON response to NVCS, which includes both the run recipe information and a link that can be used to download the sample sheet that will later be used with analysis software such as bcl2fastq2.
2. When the run starts, NVCS sends a RunStarted run status request. The Sequencer API then:
   - Validates reagent kit information in the RunStarted request:
   – Checks that the reagent kit exists. If it does not, creates it and enables it on the master step.
   – Checks that the reagent kit is activated. If it is not, activates it.
   - Starts the step for the queued sample(s). The step produces one output file placeholder per lane of the flow cell in use, based on the flow cell type.
   - Records all relevant information from the RunStarted request on the step, such as reagent lots and step fields.
3. At the end of the run, NVCS sends one of the following run status requests: RunCompletedSuccessfully, RunEndedByUSer, or RunErroredOut. The Sequencer API then:Updates the step with any new information. Currently, this includes only the status and cycle/read information.
   - Updates the step with any new information. Currently, this includes only the status and cycle/read information.

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4. Once primary analysis completes, NVCS sends a request containing the parsed run metrics. Note that NVCS sends this only when the run completed successfully, but the API does not make this assumption and will accept the request regardless of status. The Sequencer API then:
    - Records the metrics into the fields on file placeholder outputs in the LIMS.
    - Completes the step in the LIMS if the status is RunCompletedSuccessfully. For all other status options, the step remains in-progress. Note that this is the default autocomplete step behavior. For other configuration options, see the *NovaSeq 6000 Integration v3.2.1 Configuration and User Interaction* on page 798.

The *NovaSeq 6000 Integration v3.2.1 Configuration and User Interaction* on page 798. provides detailed information on how the following components work with the Sequencer API integration:

- Sample sheet generation and contents
- NovaSeq run
- User authentication and login
- Run recipe contents

Troubleshooting

If an automation trigger does not appear to run its corresponding scripts, see the following articles:

- *Troubleshooting Automated Informatics* in the Clarity LIMS documentation, Automated Informatics section
- *Troubleshooting EPP/Automation* in the API > Clarity LIMS API Overview > API Advanced Topics section

If an error occurs that does not provide direction on how to proceed, complete the following steps:

1. Confirm the version of the installed Illumina NovaSeq Integration Package by running the following command on the LIMS server command line:
    ```
    rpm -qa | grep -i novaseq
    ```
2. If the error is related to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0) step, review the log file information. See *Logging* on page 912, below.

If the automated run step starts, but does not complete:

1. Use one of the following methods to open the in-progress step in the LIMS:
    - Log in to the default user account.
    - In Lab View, find the step in the Recent Activities sidebar.
    - Search for the step in the LIMS using either the Library Tube or Flow Cell barcode as the search term, depending on whether this is a NovaSeq Standard or NovaSeq Xp run.
2. On the Record Details screen, the Sequencing Log multiline text field will contain logging information.

- If you are unable to reach the Record Details screen, or if the Sequencing Log field does not contain enough information to resolve the issue, review the sequencer-api.log file described below.

3. Contact the BaseSpace Clarity LIMS support team, supplying the relevant information from the troubleshooting steps already performed.

Additional troubleshooting information is provided on the *Illumina Instrument Integrations FAQ* on page 2598 page.

Logging

In addition to updating the Sequencing Log multiline text field on the AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0) step, the Sequencer API writes a detailed log file to /opt/gls/clarity/tomcat/current/logs/sequencer-api.log.

Log messages include the Library Tube ID and Flow Cell ID whenever the messages are related to a sequencing run-related request. In the LIMS workflows, these IDs are recorded as container names.

Some log messages are not directly related to a sequencing run request, such as downloading the sample sheet. In this case, the file LIMS ID and file name are included in log messages. The sample sheet downloaded by the sequencer will, by default, have a name that includes the sequencing container's ID (Library Tube for Standard or Flow Cell for Xp), so the relevant container name search will find the majority of the log messages for this request as well. However, if you wish to find all messages for downloading a file, then you must know the LIMS ID of the file. You can find this by looking in the LIMS API and, for sample sheets used in a run, in the recipe response (which should also be found in the log file).

Below are some example lines from the log file:

```
2018-06-19 21:32:19.705 INFO --- [] SequencerAPIApplication : Started
SequencerAPIApplication in 7.026 seconds (JVM running for 15.964)
...
2018-06-19 21:34:44.564 INFO --- [Library Tube Id=NV1234567-LIB, Flow Cell
Id=RM123DSXX] LimsApiLookupUtil : Searching for containers with name 'NV1234567-
LIB' or 'RM123DSXX'
....
2018-06-19 21:35:27.158 INFO --- [] FileService : Successfully retrieved file
'NV1234567-LIB.csv' with LIMS ID 40-51 through the API.
```

Illumina NovaSeq Integration v3.2.1 Release Notes

Compatibility

- BaseSpace Clarity LIMS v4.2 or later. Not compatible with v6.0 and later.
- NGS Extensions Package v5.18 or later.
- Pre-configured Workflow Package (PCWP) v1.2 or later.

Illumina NovaSeq Software Compatibility

- NovaSeq Control Software (NVCS) v1.6.0.

Features to Note for this Release

This release of the BaseSpace Clarity LIMS integration package provides:

- API-based integration between NovaSeq instrument and BaseSpace Clarity LIMS.
- Support for asymmetric sequencing read length.
- Support for the newly launched SPrime (SP) flowcell.
- Support for long read sequencing (2x250) on SPrime (SP) flowcell.
- Updated workflow (NovaSeq 6000 v3.0) delivered directly from PCWP v1.2
- Direct file and information exchange between Clarity LIMS and NovaSeq Control Software (NVCS) via API includes the following:
  - From LIMS to NVCS:
    - Sample sheet generated on LIMS to NVCS
    - Run recipe generated on LIMS to NVCS
  - From NVCS to LIMS:
    - Run status updates (RunStarted, RunCompletedSuccessfully, RunEndedByUser, RunErroredOut) from NVCS to LIMS
    - Run metrics information from NVCS to LIMS

Known issues

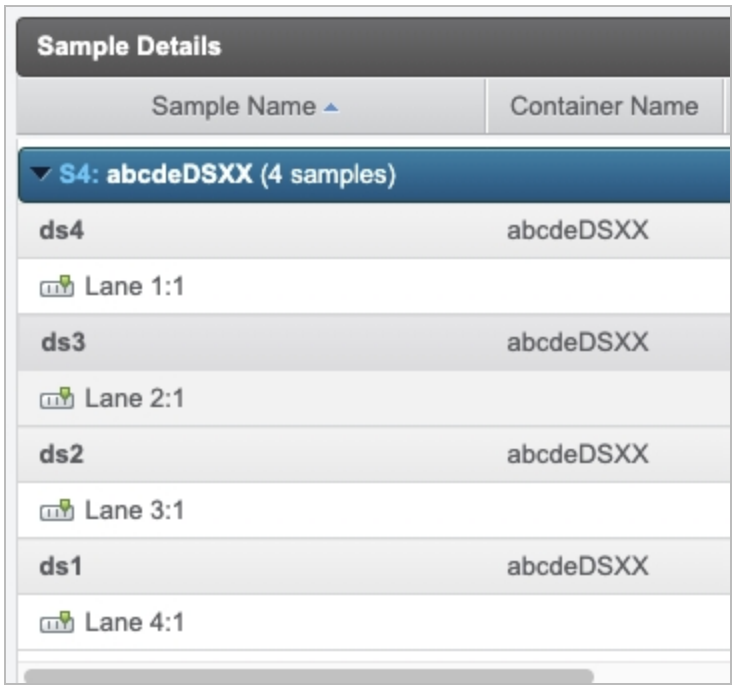
- In the run metrics payload that NVCS v1.6.0 sends to Clarity LIMS:
  - ReadsPF information is currently not available. (This will be fixed in the upcoming NVCS release.)
  - R2 error rate, R2 Q30, and R2 yield have slight inconsistency compared to values on Illumina Sequencing Analysis Viewer (SAV) software.

Migrate NovaSeq 6000 v3.0 Configuration to Display the Correct Lane Number

Recommended Usage: For users implementing the NovaSeq 6000 v3.0 workflow.

This solution provides a way to fix the incorrect lane number display without any software upgrades, by using the "InputWellLocation" token instead of "OutputItemNumber." However, it is not a perfect solution, since the sample details will display both container row and column numbers.

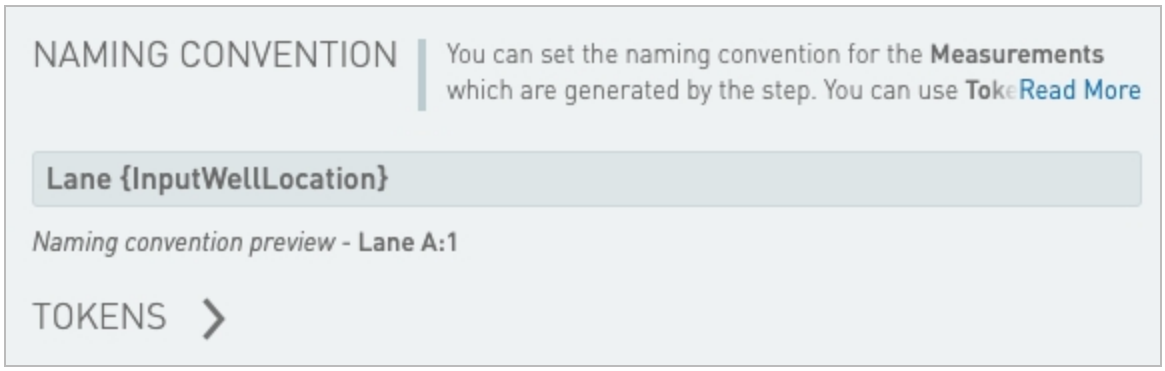
Here is an illustration of the result:



Instructions

To resolve this issue, you must update the “Naming Convention” configuration for master step: AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0).

1. Log into BaseSpace Clarity LIMS with System Administrator permissions.
2. In the Configuration area, click LAB WORK, and then choose master step AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.0).
3. In Naming Convention, change the value to `Lane {InputWellLocation}`.



4. Click Save.

NovaSeq 6000 Integrations v2

The Illumina NovaSeq 6000 instrument integration provides preconfigured workflows that map to established lab protocols and steps used with NovaSeq instruments. The integration also supports the associated library prep kits, reagent kits, and assays used in these protocols.

The documents in this section support Illumina NovaSeq 6000 Integration version 2.x.

NovaSeq 6000 Integration v2.5 Configuration & User Interaction

The Illumina NovaSeq 6000 Integration Package v2.5 supports the integration of Clarity LIMS to NovaSeq 6000 instruments.

This document describes the integration and includes information about the preconfigured protocols, steps, automations, installed components, configuration requirements, rules, and constraints.

For instructions on validating and troubleshooting the Illumina NovaSeq 6000 Integration, refer to *NovaSeq 6000 Integration v2.5 Validation and Troubleshooting* on page 972.

> The configuration provided in this integration has been established to support NovaSeq 6000 lab processes. Any configuration changes to protocols or workflows—including renaming protocols, steps, and fields—could break the process.

Integration Functionality

The integration includes the following functionality:

- Preconfigured NovaSeq 6000 v2.3 workflow that maps to lab protocols and instrument runs.
- Preconfigured NovaSeq Standard (NovaSeq 6000 v2.3) protocol that supports the loading of pooled libraries into a library tube.
- Preconfigured NovaSeq Xp (NovaSeq 6000 v2.3) protocol that supports individual lane loading on the NovaSeq.
- Automated generation of a sample sheet for use with bcl2fastq2 v2.20 analysis software.
- Automatic generation of run recipe file (JSON format) that is automatically uploaded to the sequencing instrument and used to set up and initiate the run.
- Automated tracking of the NovaSeq sequencing run and parsing of run statistics (per run per lane) into Clarity LIMS, including:
  - Progress and metrics of sequencing run

  - Per instrument sequencing runs (tracked as part of the Run ID field value)
  - Sequencing run parameters
  - Real-Time Analysis v3 (RTA3) run directory location and other run-specific information
- Preconfigured NovaSeq Validation Library Prep (NovaSeq 6000 v2.3) workflow used for validation purposes only. The workflow contains a single-step protocol that models the library prep required to produce normalized libraries that are ready for the NovaSeq 6000 v2.3 workflow. For details, refer to *NovaSeq 6000 Integration v2.5 Validation and Troubleshooting* on page 972.

Prerequisites and Assumptions

It is assumed that samples enter the NovaSeq 6000 v2.3 workflow as normalized libraries. That is, before they are assigned to the workflow the following actions occur:

- Samples have been accessioned into Clarity LIMS.
- Samples have been run through QC and library prep.
- Samples have been normalized, and the value is captured in a field called Normalized Molarity (nM).

For more information on sample accessioning, refer to *Sample Accessioning* and *Upload and Modify Samples* in the Getting Started section of the Clarity LIMS Core Functionality documentation.

You can assign samples to workflows automatically, using a routing script, or manually—from the Projects & Samples dashboard. Refer to *Assign and Process Samples* in the Clarity LIMS Core Functionality documentation.

Workflows, Protocols, and Steps

The Illumina NovaSeq 6000 Integration Package v2.5 includes two workflows:

- NovaSeq 6000 v2.3
- NovaSeq Validation Library Prep (NovaSeq 6000 v2.3)

The following table lists and describes the protocols and steps included in these workflows.

Table 217 NovaSeq 6000 Integration Package v2.5 Protocols and Steps

Protocol Name	Protocol Purpose	Protocol Steps
NovaSeq Validation Library Prep (NovaSeq 6000 v2.3) workflow		

Protocol Name	Protocol Purpose	Protocol Steps
NovaSeq Validation Library Prep (NovaSeq 6000 v2.3)	Included for validation purposes only, this protocol models the library prep steps required to advance samples to the Run Format (NovaSeq 6000 v2.3) protocol. The protocol contains a single step: NovaSeq Validation Library Prep (NovaSeq 6000 v2.3). At the end of this step, a routing script sends the samples to the first step of the NovaSeq 6000 v2.3 workflow, which is Define Run Format (NovaSeq 6000 v2.3).	NovaSeq Validation Library Prep (NovaSeq 6000 v2.3)
	NovaSeq 6000 v2.3 workflow	
Run Format (NovaSeq 6000 v2.3)	Allows for the assignment of per sample values for Loading Workflow Type, Normalized Molarity, Flowcell Type, and Final Loading Concentration (pM). • Loading Workflow Type: Select from NovaSeq Standard or NovaSeq Xp. • Normalized Molarity: Enter a value for each sample. • Flowcell Type: Select from options S1, S2, S4, and SP. • Final Loading Concentration (pM): Select from the options 225 (PCR-free workflows) or 400 (Nano workflows). Alternatively, enter a different value. Compares the Normalized Molarity value of each sample with the Minimum Molarity value. Routing script sends samples to the NovaSeq Standard or NovaSeq Xp protocol, according to the selected Loading Workflow Type. Samples with Normalized Molarity less than Minimum Molarity are removed from the workflow.	Define Run Format (NovaSeq 6000 v2.3)
NovaSeq Standard (NovaSeq 6000 v2.3)	Samples are pooled and added to the library tube in preparation for the NovaSeq run. Sample sheet and run recipe files are generated. Routing script sends library tube to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) protocol.	Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.3) Dilute and Denature (NovaSeq 6000 v2.3)

Protocol Name	Protocol Purpose	Protocol Steps
NovaSeq Xp (NovaSeq 6000 v2.3)	Samples are pooled and added to lanes on the NovaSeq flow cell. The flow cell type is determined by the option selected in the Define Run Format (NovaSeq 6000 v2.3) step. Sample sheet and run recipe files are generated. Samples are queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) protocol.	Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.3) Dilute, Denature & ExAmp (NovaSeq 6000 v2.3) Load to Flowcell (NovaSeq 6000 v2.3)
AUTOMATED - NovaSeq Run (NovaSeq6000 v2.3)	All samples complete the workflow by going through this protocol. This protocol contains one fully automated step. Do not add samples to the Ice Bucket or start the step. The integration does this action automatically.	AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3)

Validation Workflow

The NovaSeq Validation Library Prep (NovaSeq 6000 v2.3) workflow allows for validation of the system after installation is complete. For details, refer to *NovaSeq 6000 Integration v2.5 Validation and Troubleshooting* on page 972.

Step Automations

The following table lists the step automations included in the NovaSeq 6000 v2.3 workflow. Automation details are provided in the sections following the table.

For details on the validation workflow, refer to *NovaSeq 6000 Integration v2.5 Validation and Troubleshooting* on page 972.

Table 218 NovaSeq 6000 v2.3 Workflow Step Automations

Protocol	Step	Automation
1. Run Format (NovaSeq 6000 v2.3)	1. Define Run Format (NovaSeq 6000 v2.3)	• Routing Script • Set Next Steps
2. NovaSeq Standard (NovaSeq 6000 v2.3)	1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.3)	• Calculate Volumes • Set Next Step • Validate Inputs Flowcell Type and Single Pool • Validate Unique Indexes (Not used. This functionality is handled by the core Clarity LIMS configuration for pooling.)
	2. Dilute and Denature (NovaSeq 6000 v2.3)	• Generate Sample Sheet & Run Recipe • Prepare Files for NovaSeq • Routing Script • Validate Library Tube Barcode • Validate Single Input
3. NovaSeq Xp (NovaSeq 6000 v2.3)	1. Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.3)	• Calculate Volumes • Set Next Step • Validate Inputs Flowcell Type and Single Pool • Validate Unique Indexes (Not used. This functionality is handled by the core Clarity LIMS configuration for pooling.)
	2. Dilute, Denature & ExAmp (NovaSeq 6000 v2.3)	• Calculate Volumes • Set Next Step • Validate Inputs Flowcell Type
	3. Load to Flowcell (NovaSeq 6000 v2.3)	• Generate Sample Sheet & Run Recipe • Prepare Files for NovaSeq • Validate Flowcell Barcode • Validate Inputs and Selected Container
4. AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3)	1. AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3)	• Parse Run Parameters and Generate Link to Run Folder • Read InterOp Metrics • Set Next Steps • Update Lane number

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Protocol 1: Run Format (NovaSeq 6000 v2.3)

This protocol sets the Loading Workflow Type and allows you to choose the appropriate Flowcell Type and Final Loading Concentration(pM). At the end of the protocol, a routing script sends the normalized libraries to either the NovaSeq Standard (NovaSeq 6000 v2.3) or the NovaSeq Xp (NovaSeq 6000 v2.3) protocol.

This protocol contains one step: Define Run Format (NovaSeq 6000 v2.3).

Step 1: Define Run Format (NovaSeq 6000 v2.3)

Step input: NTP (normalized libraries)

Step output: None

Set Next Steps Automation

Automatically triggered on exit of the Record Details screen, this automation does the following actions:

- Sets the next step for samples to REMOVE with `nextStep = ::REMOVE::.`
- Calculates the Minimum Molarity using the following formula:

```
input.::Minimum Molarity (nM):: = (5 * input.::Final Loading Concentration
(pM)::)/1000
```

- Checks Normalized Molarity value. For samples with no Normalized Molarity value (eg, an empty value, not including 0), the automation generates an error message stating that the field cannot be empty:

```
if (!input.hasValue(::Normalized Molarity (nM)::)) { fail(::The Normalized
Molarity cannot be empty.::) ; }
```

- Compares the Normalized Molarity value of each sample with the Minimum Molarity value. If Normalized Molarity value is lower than the Minimum Molarity value, sets the Loading Workflow Type of the sample to [Remove from workflow] and records a message in the Warning field:

```
else if (input.::Normalized Molarity (nM):: < input.::Minimum Molarity (nM)::) {
input.::Warning:: = ::The Normalized Molarity is too low.:: ; input.::Loading
Workflow Type:: = ::[Remove from workflow]:: ; } else { input.::Warning:: =
::n/a:: }
```

  At this point, there are two options:

  - Correct the Normalized Molarity value on the Record Details screen. Also, edit the Loading Workflow Type field and set it to NovaSeq Standard or NovaSeq Xp, as applicable.
  - Complete the protocol without correcting the Normalized Molarity value. In this case, those samples are removed from the workflow.

Routing Script Automation

Automatically triggered on exit of the step, this automation invokes the changeWorkflow script, which routes step inputs appropriately.

- Samples with Loading Workflow Type field value of NovaSeq Standard are routed to the NovaSeq 6000 v2.3 workflow and queued for the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.3) step.
- Samples with Loading Workflow Type field value of NovaSeq Xp are routed to the NovaSeq 6000 v2.3 workflow and queued for the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.3) step.

The default automation command line is as follows.

```
bash -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar
-u {username} -p {password} -i {stepURI:v2} -l {compoundOutputFileLuid0}
script:changeWorkflow \
\
--FIELD_NAME 'Loading Workflow Type' \
--FIELD_VALUE 'NovaSeq Standard' \
--WORKFLOW 'NovaSeq 6000 v2.3' \
--STEP 'Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.3)' \
--INPUTS_OR_OUTPUTS 'INPUTS' \
\
--FIELD_NAME 'Loading Workflow Type' \
--FIELD_VALUE 'NovaSeq Xp' \
--WORKFLOW 'NovaSeq 6000 v2.3' \
--STEP 'Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.3)' \
--INPUTS_OR_OUTPUTS 'INPUTS'"
```

Master Step Fields

The following table provides field configuration details for the fields defined on the Define Run Format (NovaSeq 6000 v2.3) step.

Table 219 Define Run Format (NovaSeq 6000 v2.3) Master Step Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Comment	Multiline Text		None

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Instruction	Text	Read Only	Default • Add Flowcell Type and Loading Workflow Type below
Flowcell Type	Text Dropdown	Required Field Custom Entries	Hidden (does not show on Record Details screen) Presets • S1 • S2 • S3 • S4
Loading Workflow Type	Text Dropdown	Required Field	Hidden (does not show on Record Details screen) Presets • NovaSeq Standard • NovaSeq Xp

Global Fields

The following table lists the global fields that are configured to display on the Define Run Format (NovaSeq 6000 v2.3) step.

Table 220 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
Adjusted Per Sample Volume (µl)	Numeric	Read Only	Decimal places displayed = 2

Field Name	Field Type	Options	Additional Options and Dropdown Items
Final Loading Concentration (pM)	Numeric Dropdown	Required Field Custom Entries	Presets • 225 • 400 Decimal places displayed = 0
Flowcell Type	Text Dropdown	Required Field	Presets • S1 • S2 • S4 • SP
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
Minimum Molarity (nM)	Numeric		Decimal places displayed = 2
Normalized Molarity (nM)	Numeric		Decimal places displayed = 2
Per Sample Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Warning	Text Dropdown	Read Only Custom Entries	Presets • The Normalized Molarity (nM) is too low. • n/a

User Interaction

1. On the Record Details screen in the Sample Details table, complete the following fields (values can vary across samples):
   - **Loading Workflow Type**—Select NovaSeq Standard or NovaSeq Xp from the drop-down list.
   - **Flowcell Type**—Select S1, S2, S4, or SP from the drop-down list.
   - **Final Loading Concentration (pM)**—Select from the two preset options (225 (for PCR-free workflows) or 400 (for Nano workflows)) or enter a different value.

- **Normalized Molarity**—Enter a value for each sample.

2. Select Next Steps.
   This selection triggers the Set Next Steps automation, which does the following actions:
   - Sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step successfully.
   - Calculates the Minimum Molarity.
   - Checks Normalized Molarity value. For samples with no Normalized Molarity value (eg, an empty value, not including 0), the automation generates an error message stating that the field cannot be empty.
   - Compares the Normalized Molarity value of each sample with the Minimum Molarity value.
3. On the Assign Next Steps screen, observe the following changes:
   - In the Sample Details table, the Next Step for all samples is prepopulated with Remove from workflow—regardless of the Loading Workflow Type.

     Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.

   - For samples whose Normalized Molarity value is lower than the Minimum Molarity value, the Loading Workflow Type is set to Remove from workflow and a message is recorded in the Warning field for the sample.
4. [Optional] If the Normalized Molarity value is lower than the Minimum Molarity value, complete one of the following options:
   - Return to the Record Details screen and adjust the Normalized Molarity value so that it equals or exceeds the Minimum Molarity value. Also, set the Loading Workflow Type to NovaSeq Standard or NovaSeq Xp.
   - Complete the protocol without correcting the Normalized Molarity value. In this case, the samples in question are removed from the Clarity LIMS workflow.
5. Select Finish Step.
   The Routing Script automation is triggered when:
   - Samples where Loading Workflow Type is set to Remove from workflow (eg, where Normalized Molarity value is lower than the Minimum Molarity) are removed from the Clarity LIMS workflow.
   - Samples where Loading Workflow Type is set to NovaSeq Standard are routed to the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.3) step. This step is the first of two steps in the NovaSeq Standard (NovaSeq 6000 v2.3) protocol.
   - Samples where Loading Workflow Type is set to NovaSeq Xp are routed to the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.3) step. This step is the first of three steps in the NovaSeq Xp (NovaSeq 6000 v2.3) protocol.

Protocol 2: NovaSeq Standard (NovaSeq 6000 v2.3)

Samples are routed to this protocol if the Loading Workflow Type value is set to NovaSeq Standard. Samples are pooled and added to a library tube in preparation for the NovaSeq run.

At the end of this protocol, a routing script sends the library tube to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) protocol.

This protocol contains two steps:

1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.3)
2. Dilute and Denature (NovaSeq 6000 v2.3)

Step 1: Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.3)

In this step, manually place libraries into a single pool. Resuspension buffer and reagents are added.

> Only create one pool per step.

Step input: NTP (normalized libraries)

Step output: Bulk pool

The following automations are configured on the step:

- Calculate Volumes
- Set Next Step
- Validate Inputs Flowcell Type and Single Pool
- Validate Unique Indexes (Not used. This functionality is handled by the core Clarity LIMS configuration for pooling)

These automations are described in the order in which they are triggered at run time.

Validate Inputs Flowcell Type and Single Pool Automation

Automatically triggered on exit of the Pooling screen, this automation checks the following information:

- All samples in the pool have the same Flowcell Type assigned to them.
- Only one pool has been created.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/novaseq/novaseq-extensions.jar script:validate_flowcell_for_input_pools -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -validateSingleOutput true -poolType bulk
```

Calculate Volumes Automation

Due to the 4000-character limit, part of the script definition of Calculate Volumes has been moved into the Calculate Volumes Script field. If this part of the script needs to be modified, you can make the changes to the default value of the Calculate Volumes Script field. The parts of the script that were moved into the Calculate Volumes Script field are identified below.

Automatically triggered when the Calculate Volumes button on the Record Details screen is selected, this automation does the following actions:

- Calculates the number of samples in the pool:

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: + 1
```

- Sets the value of the Bulk Pool Volume (µl) and PhiX Volume (µl) fields, based on the selected Flowcell Type. The following part of the automation is found in the Calculate Volumes Script field:

```
step.::PhiX Volume (ul):: = !step.hasValue(::% PhiX (2.5nM) Spike-In::) ? 0 :
step.::% PhiX (2.5nM) Spike-In::;
if (input.::Flowcell Type:: == ::SP::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 100;
step.::PhiX Volume (ul):: *= 0.6
};
if (input.::Flowcell Type:: == ::S1::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 100;
step.::PhiX Volume (ul):: *= 0.6
};
if (input.::Flowcell Type:: == ::S2::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 150;
step.::PhiX Volume (ul):: *= 0.9
};
if (input.::Flowcell Type:: == ::S4::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 310;
step.::PhiX Volume (ul):: *= 1.9
};
if (step.::PhiX Volume (ul):: == 0) {
step.::PhiX Volume (ul):: = ::::
};
```

- Calculates the Per Sample Volume (µl) to be added to the pool. The following part of the automation is found in the Calculate Volumes Script field:

```
input.::Per Sample Volume (µl):: = ( ( (input.::Final Loading Concentration
(pM):: * 5 / 1000) / input.::Normalized Molarity (nM):: ) * step.::Bulk Pool
Volume (µl):: ) / step.::Number of Samples in Pool::
```


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To ensure accurate pipetting of each sample in a pool for sequencing, the Per Sample Volume (µl) value must be equal to or higher than the Minimum Per Sample Volume (µl). The default value is set at 5, which can be edited.

Assuming the default Minimum Per Sample Volume (µl) value of 5, for a given batch:

1. If the smallest Per Sample Volume (µl) value is less than 5, Clarity LIMS automatically assigns a value of 5 to the Adjusted Per Sample Volume (µl) field.
2. Clarity LIMS then adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

- Calculates the Total Sample Volume (µl) field value:

```
step.::Total Sample Volume (µl):: = step.::Total Sample Volume (µl):: +
input.::Adjusted Per Sample Volume (µl)::
```

- If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) field value:

```
if (step.::Total Sample Volume (µl):: >= step.::Bulk Pool Volume (µl)::)
{output.::RSB Volume (µl):: = 0} else {output.::RSB Volume (µl):: = step.::Bulk
Pool Volume (µl):: - step.::Total Sample Volume (µl)::} ;
```

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs:

```
output.::Flowcell Type::= input.::Flowcell Type::;output.::Loading Workflow
Type::= input.::Loading Workflow Type::;
```

- Sets the Volume of Pool to Denature (µl) value and calculates NaOH Volume (µl) and Tris-HCl Volume (µl) values, based on the Flowcell Type:

```
step.::PhiX Volume (ul):: = !step.hasValue('% PhiX (2.5nM) Spike-In') ? 0 :
step.'% PhiX (2.5nM) Spike-In';
if (input.::Flowcell Type:: == ::SP::) {
output.::Volume of Pool to Denature (ul):: = 100;
output.::NaOH Volume (ul):: = 25;
output.::Tris-HCl Volume (ul):: = 25;
}
if (input.::Flowcell Type:: == ::S1::) {
output.::Volume of Pool to Denature (ul):: = 100;
output.::NaOH Volume (ul):: = 25;
output.::Tris-HCl Volume (ul):: = 25;
}
if (input.::Flowcell Type:: == ::S2::) {
output.::Volume of Pool to Denature (ul):: = 150;
output.::NaOH Volume (ul):: = 37;
output.::Tris-HCl Volume (ul):: = 38;
```

```
}
if (input.::Flowcell Type:: == ::S4::) {
output.::Volume of Pool to Denature (ul):: = 310;
output.::NaOH Volume (ul):: = 77;
output.::Tris-HCl Volume (ul):: = 78;
}
```

- Uses the `NovaSeq_Standard_Bulk_Pool1.csv`, `NovaSeq_Standard_Bulk_Pool2.csv`, and `NovaSeq_Standard_Bulk_Pool3.csv` template files to generate a single CSV file containing information about the pool and the samples it contains. The generated file is available for download on the Step Setup screen of the following step: Dilute and Denature (NovaSeq 6000 v2.3).

```
script:driver_file_generator \ -t
/opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Standard_Bulk_Pool1.csv \
-o 1.csv \ script:driver_file_generator \ -t
/opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Standard_Bulk_Pool2.csv \
-o 2.csv \ script:driver_file_generator \ -t
/opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Standard_Bulk_Pool3.csv \
-o 3.csv \&& cat 1.csv 2.csv 3.csv > {compoundOutputFileLuid0}.csv
```

- Resets the Total Sample Volume (ul) and Number of Samples in Pool field values so that the automation is idempotent:

```
step.'Number of Samples in Pool' = step.'Number of Samples in Pool' - 1;
step.'Total Sample Volume (ul)' = step.'Total Sample Volume (ul)' -
input.'Adjusted Per Sample Volume (ul)'
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, the following automation sets the next step for samples to ADVANCE, advancing them to the Dilute and Denature (NovaSeq 6000 v2.3) step in the protocol:

```
nextStep = ::ADVANCE::
```

Master Step Fields

The following table provides field configuration details for the fields defined on the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.3) step.

Table 221 Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.3) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Drop-Down Items
% PhiX (2.5nM) Spike-In	Numeric		Range = 1–100 Decimal places displayed = 0
Bulk Pool Volume (µl) (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 2
Minimum Per Sample Volume (uL)	Numeric	Required Field	Default • 5 Decimal places displayed = 2
Number of Flowcells to Sequence	Numeric	Required Field	Range = 1–10 Decimal places displayed = 0
Number of Samples in Pool (for calculation purposes, not displayed)	Numeric		Default • 0 Decimal places displayed = 0
PhiX Volume (µl) (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 2
Total Sample Volume (µl) (for calculation purposes, not displayed)	Numeric		Default • 0 Decimal places displayed = 2
Calculate Volumes Script	Text	Required Field Read Only	Default value provided in the drop-down section following the table. Do not remove this field. It is used by the Calculate Volumes automation script.

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Calculate Volumes Script

```
step.::PhiX Volume (ul):: = !step.hasValue(::% PhiX (2.5nM) Spike-In::) ? 0 :
step.::% PhiX (2.5nM) Spike-In::; if (input.::Flowcell Type:: == ::SP::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 100;
step.::PhiX Volume (ul):: *= 0.6 }; if (input.::Flowcell Type:: == ::S1::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 100;
step.::PhiX Volume (ul):: *= 0.6 }; if (input.::Flowcell Type:: == ::S2::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 150;
step.::PhiX Volume (ul):: *= 0.9 }; if (input.::Flowcell Type:: == ::S4::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 310;
step.::PhiX Volume (ul):: *= 1.9 }; if (step.::PhiX Volume (ul):: == 0) {
step.::PhiX Volume (ul):: = :::: }; input.::Per Sample Volume (ul):: =
(((input.::Final Loading Concentration (pM):: * 5 / 1000) / input.::Normalized
Molarity (nM)::) * step.::Bulk Pool Volume (ul)::) / step.::Number of Samples in
Pool::
```

Global Fields

The following table lists the global fields that are configured to display on the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.3) step.

Table 222 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Drop-Down Items
Configured on Derived Sample			
Flowcell Type	Text Dropdown	Required Field	Presets • S1 • S2 • S4 • SP
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
NaOH Volume (µl)	Numeric	Read Only	Decimal places displayed = 2

Field Name	Field Type	Options	Additional Options and Drop-Down Items
RSB Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Tris-HCl Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Volume of Pool to Denature (µl) (Used in Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.3) step only. Displays on Record Details screen and in the generated CSV file.)	Numeric	Read Only	Decimal places displayed = 0

User Interaction

1. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucket and select View Ice Bucket.
2. On the Ice Bucket screen, select Begin Work.
3. On the Pooling screen, create a pool as follows.
   a. Create a pool by dragging samples into the Pool Creator.
   b. Name the pool or accepts the default name (Pool #1).
   c. Select Record Detail.

   On exit from the Pooling screen, the Validate Inputs Flowcell Type and Single Pool is triggered. The automation checks that all samples in the pool have been assigned the same Flowcell Type and only one pool has been created.
4. On the Record Details screen in the Step Details area, complete the fields as follows.
   a. Enter the value for the Number of Flowcells to Sequence field.
      This value is used in volume calculations to make sure that the volumes are sufficient for the number of times the pool is sequenced.
   b. Enter the value for the Minimum Per Sample Volume (µl) field.
      The value in this field is used to calculate how much of each sample is included in the pool. The field is prepopulated with the configured default value (5 µl), but the value can be edited if necessary.
   c. If necessary, enter the value for the % PhiX (2.5 nM) Spike-In field.

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The value in this field is used to calculate the volume of PhiX v3 control to be included in the pool for the given percentage of spike-in. The field is optional and can be edited.

5. In the Sample Details table, select the pool icon to view details on the pool composition.
6. Select Calculate Volumes.
   This selection triggers the Calculate Volumes automation, which performs the following volume calculations based on the selected Flowcell Type:
   - Calculates Bulk Pool Volume (µl) value.
   - Calculates PhiX Volume (µl) value if % PhiX (2.5 nM) Spike-In has value.
   - Calculates the Per Sample Volume (µl) value to be added to the pool.
   - Calculates the Total Sample Volume (µl) value.
   - If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) value.
   - Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
   - Populates the Volume of Pool to Denature (µl), NaOH Volume (µl), and Tris-HCl Volume (µl) columns of the Sample Details table. (Values are set by a script and are not editable.)
   - Generates the Calculation File (CSV) and attaches it to the step. This file contains volume information about the pool and the individual samples it contains. Select the file to download it, then open it in Excel.
7. Select Next Steps.
   This selection triggers the Set Next Step automation. This automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol: Dilute and Denature (NovaSeq 6000 v2.3).
   On the Assign Next Steps screen, the next step is already set to Dilute and Denature (NovaSeq 6000 v2.3).
8. Select Finish Step.
   At the end of this step, the pool of samples automatically advances to the Dilute and Denature (NovaSeq 6000 v2.3) step.

Step 2: Dilute and Denature (NovaSeq 6000 v2.3)

In this step, pooled samples are denatured and diluted by the addition of NaOH, Tris-HCl, and Resuspension Buffer (RSB). Samples are manually placed into the library tube that is used in the NovaSeq run. In addition, this step generates the sample sheet file and the run recipe (*json file) needed to start the NovaSeq run. The step input is Bulk pool, and the step output is Library tube.

The following automations are configured on the step:

- Generate Sample Sheet & Run Recipe
- Prepare Files for NovaSeq
- Routing Script

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- Validate Library Tube Barcode
- Validate Single Input

Validate Single Input Automation

Automatically triggered at the beginning of the step, the following automation checks that there is only one container input to the step:

```
script:validateSampleCount -min 1 -max 1
```

Validate Library Tube Barcode Automation

Automatically triggered on exit of the Placement screen, the following automation validates the library tube barcode to make sure it conforms to the barcode mask [A-Z]{2}[0-9]{7}-[A-Z]{3}:

```
if (!output.container.name.matches(::[A-Z]{2}[0-9]{7}-[A-Z]{3}::)
fail(::Invalid Library Tube Barcode. Please verify and try again.::)
}
```

Generate Sample Sheet & Run Recipe Automation

Automatically triggered when a button on the Record Details screen is selected, this automation does the following actions:

- Copies the Flowcell Type from the step input to the step output:

```
output.::Flowcell Type:: = input.::Flowcell Type::
```

- Copies the Flowcell Type from the step input to the Run Mode field (hidden):

```
step.::Run Mode:: = input.::Flowcell Type::
```

- Copies the Loading Workflow Type values from the step inputs to the step outputs:

```
output.::Loading Workflow Type:: = input.::Loading Workflow Type::;
```

- Validates the parameters entered on the Record Details screen:
  - Experiment Name can only contain alphanumeric, dash, or underscore characters. Spaces are not permitted.
  - When Workflow Type is No Index, Index Read 1 must be zero. For any other Workflow Type, Index Read 1 must be greater than zero.

```
if(!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) {
fail(::Experiment Name contains prohibited characters. Allowed characters are:
a-z, A-Z, 0-9, -, and _::)
}
if(step.::Workflow Type::== ::No Index::) {
if(step.::Index Read 1::!= 0) {
fail(::Index Read 1 must be 0 if the Workflow Type is No Index.::)
}
```


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```
} else {
if(step.::Index Read 1::== 0) {
fail(::Index Read 1 must be greater than 0 if the Workflow Type is ::+
step.::Workflow Type::+ ::.::)
}
}
```

- When Paired End is True, Index Read 1 and Index Read 2 must be greater than zero.
- When Paired End is False, Index Read 2 must be zero, Reverse Complement Workflow must be false, UMI - Read 2 Length must not have a value, and UMI - Read 2 Start from Cycle must not have a value.

```
if (step.::Paired End::.toBoolean()) {
if (step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) {
fail(::Read 1 Cycles and Read 2 Cycles must be greater than 0 if Paired End is
True.::);
}
} else {
if (step.::Read 2 Cycles:: != 0) {
fail(::Read 2 Cycles must be 0 if Paired End is False.::);
}
if (step.hasValue(::UMI - Read 2 Length::) || step.hasValue(::UMI - Read 2 Start
From Cycle::)) {
fail(::UMI - Read 2 Length and UMI - Read 2 Start From Cycle cannot be defined
if Paired End is False.::);
}
}
```

- Validates allowed read cycles to be not greater than 151 if the Flowcell Type is not SP.

```
if (input.::Flowcell Type:: != ::SP:: && step.::Read 1 Cycles:: > 151) {
fail(::Read 1 Cycles must not be larger than 151 if it is not SPrime
Flowcell.::);
}
if (input.::Flowcell Type:: != ::SP:: && step.::Read 2 Cycles:: > 151) {
fail(::Read 2 Cycles must not be larger than 151 if it is not SPrime
Flowcell.::);
}
```

- Validates allowed value for UMI - Read 1 Length, UMI - Read 2 Length, UMI - Read 1 From Cycle, UMI - Read 2 From Cycle.

```
if (step.hasValue(::UMI - Read 1 Length::) && !step.hasValue(::UMI - Read 1
Start From Cycle::)) {
```


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```
fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 1
Length is greater than 0.::);
}
if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 1
Start From Cycle::)) {
fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 1 Start From
Cycle is greater than 0.::);
}
if (step.hasValue(::UMI - Read 2 Length::) && !step.hasValue(::UMI - Read 2
Start From Cycle::)) {
fail(::UMI - Read 2 Start From Cycle must be greater than 0 if UMI - Read 2
Length is greater than 0.::);
}
if (!step.hasValue(::UMI - Read 2 Length::) && step.hasValue(::UMI - Read 2
Start From Cycle::)) {
fail(::UMI - Read 2 Length must be greater than 0 if UMI - Read 2 Start From
Cycle is greater than 0.::);
}
if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 2
Length::)) {
fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 2 Length is
greater than 0.::);
}
if (!step.hasValue(::UMI - Read 1 Start From Cycle::) && step.hasValue(::UMI -
Read 2 Start From Cycle::)) {
fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 2
Start From Cycle is greater than 0.::);
}
```

- Sets the next step for samples to REMOVE.

```
nextStep = ::REMOVE::
```

- Generates the sample sheet and the run recipe file and attaches them to the step. For details on how the files are generated and their contents, refer to *Sample Sheet and Run Recipe File Generation* on page 969.

Prepare Files for NovaSeq Automation

Automatically triggered on exit of the Record Details screen, this automation invokes the `copy_attachments_to_network_folder` script, which is included in the `novaseq-remote-extensions.jar` file. This script places the generated sample sheet and run recipe (*json file) into the appropriate folder on the NAS, where the instrument uses them to start the run.

For more information, refer to *Sample Sheet and Run Recipe File Generation* on page 969.

Routing Script Automation

Automatically triggered on exit of the step, this automation invokes the changeWorkflow script, which routes step outputs to the NovaSeq 6000 v2.3 workflow, and queues them for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) step.

The default automation command line is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -u {username} -p {password} -i {stepURI:v2} -l {compoundOutputFileLuid2} script:changeWorkflow \
\
--FIELD_NAME 'N/A' \
--FIELD_VALUE 'N/A' \
--WORKFLOW 'NovaSeq 6000 v2.3' \
--STEP 'AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3)' \
--INPUTS_OR_OUTPUTS 'OUTPUTS'"
```

Master Step Fields

The following table provides field configuration details fields defined on the Dilute and Denature (NovaSeq 6000 v2.3) step. These fields are required for sample sheet and JSON file generation.

Table 223 Dilute and Denature (NovaSeq 6000 v2.3) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Drop-Down Items
BaseSpace Sequence Hub Configuration	Text Dropdown	Required Field	Presets • Not Used • Run Monitoring Only • Run Monitoring and Storage
Experiment Name	Text	Required Field	
Index Read 1	Numeric Dropdown	Required Field Custom Entries	Range = 0–20 Decimal places displayed = 0 Presets • 0 • 6 • 8

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Field Name	Field Type	Options	Additional Options and Drop-Down Items
Index Read 2	Numeric Dropdown	Required Field Custom Entries	Range = 0–20 Decimal places displayed = 0 Presets • 0 • 6 • 8
Paired End	Text Dropdown	Required Field	Presets • True • False
Read 1 Cycles	Numeric Dropdown	Required Field Custom Entries	Range = 1–251 Decimal places displayed = 0 Presets • 251* • 151 • 101 • 51 *The value of 251 is only supported for SP flow cell type. For all other flow cell types, the maximum value is 151.
Read 2 Cycles	Numeric Dropdown	Required Field Custom Entries	Range = 0–251 Decimal places displayed = 0 Presets • 251* • 151 • 101 • 51 *The value of 251 is only supported for SP flow cell type. For all other flow cell types, the maximum value is 151.

Field Name	Field Type	Options	Additional Options and Drop-Down Items
Reverse Complement Workflow	Toggle Switch	Required Field	Default • Yes
Run Mode (not displayed in user interface)	Text Dropdown	Read Only	Presets • SP • S1 • S2 • S4
Sample Sheet Path	Text	Read Only	
UMI - Read 1 Length	Numeric		Range = 1 Decimal places displayed = 0
UMI - Read 1 Start From Cycle	Numeric		Range = 1 Decimal places displayed = 0
UMI - Read 2 Length	Numeric		Range = 1 Decimal places displayed = 0
UMI - Read 2 Start From Cycle	Numeric		Range = 1 Decimal places displayed = 0
Use Custom Index Read 1 Primer	Toggle Switch		Default • No
Use Custom Read 1 Primer	Toggle Switch		Default • No
Use Custom Read 2 Primer	Toggle Switch		Default • No
Workflow	Text	Read Only	Default • GenerateFASTQ
Workflow Type	Text Dropdown	Required Field	Presets • No Index • Single Index • Dual Index

Global Step Fields

The following table lists the global step fields that are configured to display on the Dilute and Denature (NovaSeq 6000 v2.3) step.

Table 224 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Drop-Down Items
Configured on Derived Sample			
Flowcell Type	Text Dropdown	Required Field	Presets • S1 • S2 • S4 • SP
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User interaction

1. On the Placement screen, move the pool as follows.
   a. Drag the pool into the library tube in the Placed Samples area.
   b. Scan or types the barcode of the library tube into the Library Tube field.
   c. Select Record Details.
   On exit of the Placement screen, the Validate Library Tube Barcode automation checks that the library tube barcode conforms to the barcode mask [A-Z]{2}[0-9]{7}-[A-Z]{3}. If not, an error message displays.
2. On the Record Details screen:
   - The Reagent Lot Tracking section tracks the NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. Select from the active lots displayed in each drop-down list.

     To make reagent lots available for selection in the Reagent Lot Tracking section, configure and activate them. Do this step on the Configuration > Consumables screen.

   - The fields displayed in the Step Details section are used to generate the sample sheet and run recipe files. Some of these fields are autopopulated and some must be completed manually (refer to the following table for details).

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Table 225 Fields Displayed on Record Details Screen of Dilute and Denature (NovaSeq 6000 v2.3) Step

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.
BaseSpace Sequence Hub Configuration	Presets • Not Used • Run Monitoring Only • Run Monitoring and Storage
Workflow	Presets • GenerateFASTQ
Workflow Type	Presets • No Index • Single Index • Dual Index
Index Read 1	Presets • 0 • 6 • 8 • type a value between 0 and 20
Index Read 2	Presets • 0 • 6 • 8 • type a value between 0 and 20
Paired End	Presets • True • False
Read 1 Cycles	Presets • 251 • 151 • 101 • 51 • Type a value between 1 and 251 for the SP flow cell type or between 1 and 151 for all other types

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Field	Value
Read 2 Cycles	Presets • 251 • 151 • 101 • 51 • Type a value between 0 and 251 for the SP flow cell type or between 0 and 151 for all other types
Use Custom Read 1 Primer	Select if applicable.
Use Custom Read 2 Primer	Select if applicable.
Use Custom Index Read 1 Primer	Select if applicable.
Reverse Complement Workflow	Select Yes (by default) for v1.5 reagents in NovaSeq Control Software v1.7 and onwards. Select No for v1 reagents.
UMI - Read 1 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 1. Leave blank if not applicable.
UMI - Read 2 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 2. Leave blank if not applicable.
UMI - Read 1 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.
UMI - Read 2 Start From Cycle	Enter the cycle number that Read 2 starts from. Leave blank if not applicable.
Sample Sheet Path	Automatically populated after the sample sheet has been generated.

3. Select Generate Sample Sheet & Run Recipe.
   This selection triggers the automation script, which does the following actions:
   - Generates the sample sheet and JSON files and attaches them to the placeholders in the Files area of the Record Details screen.
   - Populates the Sample Sheet Path field with the path to the sample sheet file.
   - Sets the value of the next step to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step successfully.
4. Select Next Steps.
   This selection triggers the Prepare Files for NovaSeq automation, which copies the sample sheet and run recipe files to the location specified during installation.

5. On the Assign Next Steps screen, make sure the Next Step for all samples is prepopulated with Remove from workflow.

   Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.

6. Select Finish Step.
   This will trigger the Routing Script automation and route samples to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) protocol. In Lab View, the pool of samples is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) step.

At this point in the workflow, the user interaction ends. The integration automatically starts the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) step and data from the run is parsed back into Clarity LIMS.

Protocol 3: NovaSeq Xp (NovaSeq 6000 v2.3)

Samples are routed to this protocol if the Loading Workflow Type value is set to NovaSeq Xp.

Samples are pooled and added to lanes on the NovaSeq flow cell type selected in the Define Run Format (NovaSeq 6000 v2.3) step.

At the end of the protocol, a routing script sends the flow cell to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) protocol.

This protocol contains three steps:

1. Step 1: Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.3)
2. Step 2: Dilute, Denature & ExAmp (NovaSeq 6000 v2.3)
3. Step 3: Load to Flowcell (NovaSeq 6000 v2.3)

Step 1: Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.3)

In this step, the libraries are manually placed into a pool. The step input is NTP (normalized libraries) and the step output is Bulk pool.

Only create one pool per step.

The following automation is configured on the step:

- Calculate Volumes
- Set Next Step
- Validate Inputs Flowcell Type and Single Pool
- Validate Unique Indexes (Not used. This functionality is handled by the core Clarity LIMS configuration for pooling.)

These automations are described in the order in which they are triggered at run time.

Validate Inputs Flowcell Type and Single Pool Automation

Automatically triggered on exit of the Pooling screen, this automation checks the following information:

- All samples in the pool have the same Flowcell Type assigned to them.
- Only one pool has been created.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/novaseq/novaseq-
extensions.jar script:validate_flowcell_for_input_pools
-i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -
validateSingleOutput true
```

Calculate Volumes Automation

Automatically triggered when the Calculate Volumes button on the Record Details screen is selected, this automation:

- Calculates the number of samples in the pool:

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: + 1
```

- Sets the value of the Bulk Pool Volume (µl) and PhiX Volume (µl) field, based on the selected Flowcell Type:

```
step.::PhiX Volume (µl):: = !step.hasValue(::% PhiX (0.25nM) Spike-In::) ? 0 :
step.::% PhiX (0.25nM) Spike-In::;
if (input.::Flowcell Type:: == ::SP::) {
step.::Bulk Pool Volume (µl):: = step.'Number of Lanes to Sequence' * 18;
step.::PhiX Volume (µl):: *= 0.7;
}
if (input.::Flowcell Type:: == ::S1::) {
step.::Bulk Pool Volume (µl):: = step.'Number of Lanes to Sequence' * 18;
step.::PhiX Volume (µl):: *= 0.7;
}
if (input.::Flowcell Type:: == ::S2::) {
step.::Bulk Pool Volume (µl):: = step.'Number of Lanes to Sequence' * 22;
step.::PhiX Volume (µl):: *= 0.8;
}
if (input.::Flowcell Type:: == ::S4::) {
step.::Bulk Pool Volume (µl):: = step.'Number of Lanes to Sequence' * 30;
step.::PhiX Volume (µl):: *= 1.1;
}
if (step.::PhiX Volume (µl):: == 0) {
step.::PhiX Volume (µl):: = '';
}
```

- Calculates the Per Sample Volume (µl) to be added to the pool:

```
input.::Per Sample Volume (µl)::= (((input.::Final Loading Concentration (pM)::*
5/ 1000) / input.::Normalized Molarity (nM)::) * step.::Bulk Pool Volume (µl)::)
/ step.::Number of Samples in Pool::
```

> To ensure accurate pipetting of each sample in a pool for sequencing, the Per Sample Volume (µl) value must be equal to or higher than the Minimum Per Sample Volume (µl). The default value set at 5 and can be edited.
>
> Assuming the default Minimum Per Sample Volume (µl) value of 5, for a given batch:
>
> 1. If the smallest Per Sample Volume (µl) value is less than 5, Clarity LIMS automatically assigns a value of 5 to the sample's Adjusted Per Sample Volume (µl) field.
> 2. Clarity LIMS then adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

- Calculates the Total Sample Volume (µl) field value:

```
step.::Total Sample Volume (µl):: = step.::Total Sample Volume (µl):: +
input.::Adjusted Per Sample Volume (µl)::
```

- If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) field value:

```
if (step.::Total Sample Volume (µl):: >= step.::Bulk Pool Volume (µl)::)
{output.::RSB Volume (µl):: = 0} else {output.::RSB Volume (µl):: = step.::Bulk
Pool Volume (µl):: - step.::Total Sample Volume (µl)::} ;
```

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs:

```
output.::Flowcell Type::= input.::Flowcell Type::;
output.::Loading Workflow Type::= input.::Loading Workflow Type::;
```

- Uses the `NovaSeq_Xp_Bulk_Pool.csv` and `NovaSeq_Xp_Bulk_Pool2.csv` template files to generate a single CSV file containing information about the bulk pool and the samples it contains. The generated file is available for download on the Step Setup screen of the following step—Dilute, Denature & ExAmp (NovaSeq 6000 v2.3).
- Resets the Total Sample Volume (ul) and Number of Samples in Pool field values so that the automation is idempotent:

```
step.'Number of Samples in Pool' = step.'Number of Samples in Pool' - 1;
step.'Total Sample Volume (ul)' = step.'Total Sample Volume (ul)' -
input.'Adjusted Per Sample Volume (ul)'
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation does the following actions:

- Copies the Flowcell Type values from the step inputs to the step outputs.
- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ; nextStep = ::ADVANCE::
```

Master Step Fields

The following table provides field configuration details for the fields defined on the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.3) step.

Table 226 Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.3) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Drop-Down Items
% PhiX (0.25nM) Spike-In	Numeric		Range = 0–100 Decimal places displayed = 0
Bulk Pool Volume (µl) (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 2
Minimum Per Sample Volume (µl)	Numeric	Required Field	Default • 5 Decimal places displayed = 2
Number of Lanes to Sequence	Numeric	Required Field	Decimal places displayed = 0
Number of Sample in Pool (for calculation purposes, not displayed)	Numeric		Default • 0 Decimal places displayed = 0
PhiX Volume (µl) (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 2
Total Sample Volume (µl) (for calculation purposes, not displayed)	Numeric		Default • 0 Decimal places displayed = 0

Global Fields

The following table lists the global fields that are configured to display on the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.3) step.

Table 227 Global Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Configured on Derived Sample			
RSB Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Flowcell Type	Text Dropdown	Required Field	Presets • S1 • S2 • S4 • SP
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User Interaction

1. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucket and select View Ice Bucket.
2. On the Ice Bucket screen, select Begin Work.
3. On the Pooling screen, create a pool as follows.
   a. Drag samples into the Pool Creator to create a pool.
   b. Name the pool or accept the default name Pool #1.
   c. Select Record Details.

   On exit from the Pooling screen, the Validate Inputs Flowcell Type and Single Pool is triggered. The automation checks that all samples in the pool have been assigned the same Flowcell Type and only one pool has been created.
4. On the Record Details screen in the Step Details area, complete the fields as follows.
   a. Enter the value for the Number of Lanes to Sequence field.
      This value is used in volume calculations to make sure that the volumes are sufficient for the number of times the pool is sequenced.
   b. Enter the value for the Minimum Per Sample Volume (µl) field.

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The value in this field is used to calculate how much of each sample is included in the pool. The field is prepopulated with the configured default value (5 µl), but the value can be edited if necessary.

c. If necessary, enter the value for the % PhiX (0.25 nM) Spike-In field.
The value in this field is used to calculate the volume of PhiX v3 control to be included in the pool for the given percentage of spike-in. The field is optional and can be edited.

5. In the Sample Details table, select the pool icon to view details on the pool composition.
6. Select Calculate Volumes.
This selection triggers the Calculate Volumes automation, which performs the following volume calculations based on the selected Flowcell Type:
   - Calculates Bulk Pool Volume (µl) value.
   - If % PhiX (0.25nM) Spike-In has a value, calculates the PhiX Volume (µl) value.
   - Calculates the Per Sample Volume (µl) value to be added to the pool.
   - Calculates the Total Sample Volume (µl) value.
   - If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) value.
   - Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
   - Generates the Calculation File (*csv) and attaches it to the step. This file contains volume information about the samples and RSB buffer to add to the pool. Select the file to download it, then open it in Excel.
7. Select Next Steps.
This selection triggers the Set Next Step automation. This automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol—Dilute, Denature & ExAmp (NovaSeq 6000 v2.3).
On the Assign Next Steps screen, the next step is already set to Dilute, Denature & ExAmp (NovaSeq 6000 v2.3).
8. Select Finish Step.
At the end of this step, the pool of samples automatically advances to the Dilute, Denature & ExAmp (NovaSeq 6000 v2.3) step.

Step 2: Dilute, Denature & ExAmp (NovaSeq 6000 v2.3)

In this step, pooled samples are denatured and diluted by the addition of DPX, NaOH, Tris-HCl, and RSB. Manually create working pools based on the number of lanes you want to sequence. The step input is Bulk pool and the step output is Working pool. Working pool is a variable number for how many working pools need to be created per bulk pool.

The following automations are configured on the step:

- Calculate Volumes

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- Set Next Step
- Validate Inputs Flowcell Type

These automations are described in the order in which they are triggered at run time.

Validate Inputs Flowcell Type Automation

Automatically triggered at the beginning of the step, this automation invokes the `validate_flowcell_for_input_pools` script. This script does the following actions:

- Checks the inputs to the step and validates that the Flowcell Type field has been set to a valid value (SP, S1, S2, or S4). The automation also checks that all inputs have the same value.
- Validates that the number of outputs matches the number of lanes on the selected flow cell type. If validation fails, an error message state that the number of working pools does not match the number of lanes available on the flow cell.

```
bash -l -c "/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/novaseq/novaseq-extensions.jar script:validate_
flowcell_for_input_pools -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1}"
```

Calculate Volumes Automation

Automatically triggered on entry to of the Record Details screen, this automation sets the value of the following fields, based on the Flowcell Type:

- BP Aliquot Volume (µl)
- NaOH Volume (µl)
- Tris-HCl Volume (µl)
- DPX1 Volume (µl)
- DPX2 Volume (µl)
- DPX3 Volume (µl)
- Mastermix per Lane (µl)

```
if (input.::Flowcell Type:: == ::SP::) {
output.::BP Aliquot Volume (ul):: = 18;
output.::NaOH Volume (ul):: = 4;
output.::Tris-HCl Volume (ul):: = 5;
step.::DPX1 Volume (ul):: = 126;
step.::DPX2 Volume (ul):: = 18;
step.::DPX3 Volume (ul):: = 66;
output.::Mastermix per Lane (ul):: = 63
};
```


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```
if (input.::Flowcell Type:: == ::S1::) {
output.::bp Aliquot Volume (µl):: = 18;
output.::NaOH Volume (µl):: = 4;
output.::Tris-HCl Volume (µl):: = 5;
step.::DPX1 Volume (µl):: = 126;
step.::DPX2 Volume (µl):: = 18;
step.::DPX3 Volume (µl):: = 66;
output.::Mastermix per Lane (µl):: = 63
};
if (input.::Flowcell Type:: == ::S2::) {
output.::bp Aliquot Volume (µl):: = 22;
output.::NaOH Volume (µl):: = 5;
output.::Tris-HCl Volume (µl):: = 6;
step.::DPX1 Volume (µl):: = 126;
step.::DPX2 Volume (µl):: = 18;
step.::DPX3 Volume (µl):: = 66;
output.::Mastermix per Lane (µl):: = 77
};
if (input.::Flowcell Type:: == ::S4::) {
output.::bp Aliquot Volume (µl):: = 30;
output.::NaOH Volume (µl):: = 7;
output.::Tris-HCl Volume (µl):: = 8;
step.::DPX1 Volume (µl):: = 315;
step.::DPX2 Volume (µl):: = 45;
step.::DPX3 Volume (µl):: = 165;
output.::Mastermix per Lane (µl):: = 105
};
```

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs:

```
output.::Flowcell Type::= input.::Flowcell Type::;
output.::Loading Workflow Type::= input.::Loading Workflow Type::;
```

- Uses the `NovaSeq_Xp_Working_Pool.csv` and `NovaSeq_Xp_Working_Pool2.csv` template files to generate a single CSV file containing information about the DPX volume and the volume of BP Aliquot, Mastermix, NaOH, and Tris-HCl to add per working pool. The generated file is available for download on the Step Setup screen of the following step—Load to Flowcell (NovaSeq 6000 v2.3).

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation:

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- Copies the Flowcell Type values from the step inputs to the step outputs.
- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ; nextStep = ::ADVANCE::
```

Master Step Fields

The following table provides field configuration details for the fields defined on Dilute, Denature & ExAmp (NovaSeq 6000 v2.3) step. These field values are set by a script and are not editable while running the step.

Table 228 Dilute, Denature & ExAmp (NovaSeq 6000 v2.3) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Drop-Down Items
DPX1 Volume (µl)	Numeric	Read Only	Decimal places displayed = 0
DPX2 Volume (µl)	Numeric	Read Only	Decimal places displayed = 0
DPX3 Volume (µl)	Numeric	Read Only	Decimal places displayed = 0

Global Fields

The following table lists the global fields that are configured to display on the Dilute, Denature & ExAmp (NovaSeq 6000 v2.3) step.

Table 229 Global Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Configured on Derived Sample			
BP Aliquot Volume (µl)	Numeric	Read Only	Decimal places displayed = 0
Flowcell Type	Text Dropdown	Required Field	Presets • SP • S1 • S2 • S4

For Research Use Only. Not for use in diagnostic procedures.

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
Mastermix per Lane (µl)	Numeric	Read Only	Decimal places displayed = 0
NaOH Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Tris-HCl Volume (µl)	Numeric	Read Only	Decimal places displayed = 2

User Interaction

1. On the Queue screen, add the pool to the Ice Bucket and select View Ice Bucket.
2. On the Ice Bucket screen, set the number of derivatives to create (they are placed into the flow cell lanes) and select Begin Work.
   On entry to the Record Details screen, the Calculate Volumes automation is triggered.
   This automation sets the following values based on the selected Flowcell Type (calculation details are provided in the previous Calculate Volumes Automation section):
   - BP Aliquot Volume (µl)
   - NaOH Volume (µl)
   - Tris-HCl Volume (µl)
   - DPX1 Volume (µl)
   - DPX2 Volume (µl)
   - DPX3 Volume (µl)
   - Mastermix per Lane (µl)

   The automation also generates the Calculation File (*csv) and attaches it to the step. This file contains information about the DPX Mastermix volume and the volume of Mastermix, NaOH, and Tris-HCl to add per working pool. Select the file to download it, then open it in Excel.
3. On the Record Details screen, the Reagent Lot Tracking section tracks the DPX1, DPX2, DPX3, NaOH, RSB, and Tris-HCl reagent lots. Select from the active lots displayed in each drop-down list.

> To make reagent lots available for selection in the Reagent Lot Tracking section, configure and activate them. Do this step on the Configuration > Consumables screen.

4. In the Step Details area, the DPX1, DPX2, and DPX3 reagent volume values are already populated. (These values are set by a script and are not editable while running the step.)
5. In the Sample Details table, select the pool icon to view details on the working pool composition.
   - The BP Aliquot, Mastermix per lane, NaOH, and Tris-HCl volume values for each working pool are already populated. (These values are set by a script and are not editable while running the step.)
   - The working pool number is appended to the bulk pool name. This feature helps quickly identify which working pools are derived from the same bulk pool.
6. In the Files area, select the Calculation File (*csv) file to download it. Open the file in Excel to view information about the DPX Mastermix volume and the volume of Mastermix, NaOH, and Tris-HCl to add per working pool.
7. Select Next Steps to trigger the Set Next Step automation.
   On the Assign Next Steps screen, the next step is already set to Load to Flowcell (NovaSeq 6000 v2.3).
8. Select Finish Step.

Step 3: Load to Flowcell (NovaSeq 6000 v2.3)

In this step, scan the flow cell barcode into Clarity LIMS, then manually place the working pools into the lanes of the flow cell that is used in the NovaSeq run. The step input is Working pool and the step output is Flow cell (output containers: S1 and S2 with 2 lanes, and S4 with 4 lanes).

The following automations are configured on this step:

- Generate Sample Sheet & Run Recipe
- Prepare Files for NovaSeq
- Validate Flowcell Barcode
- Validate Inputs and Selected Container

These automations are described in the order in which they are triggered at run time.

Validate Inputs and Selected Container Automation

Automatically triggered at the beginning of the step, this automation invokes the `validate_flowcell_for_input_pools` and `validateSelectedContainer` scripts. These scripts validate the step inputs and the container selected, as follows.

- Check that the Flowcell Type field has been set to a valid value (S1, S2, S4, or SP) and that all inputs have the same value.

- Check that the number of outputs matches the number of lanes on the selected flow cell type. If validation fails, an error message states that the number of working pools does not match the number of lanes available on the flow cell. The following table defines the number of samples allowed:

Flowcell Type	Number of Working Pools
SP	2
S1	2
S2	2
S4	4

- Check that the container type selected on entry to the Placement screen matches the value in the Flowcell Type field. If validation fails, an error message displays.

Validate Flowcell Barcode Automation

Automatically triggered on exit of the Placement screen, this automation validates the flow cell barcode scanned into Clarity LIMS, using the following logic:

```
if (input.::Flowcell Type:: == ::SP:: && !output.container.name.matches(::[A-Z0-9]{5}DR[A-Z0-9]{2}::)) {
fail(::Invalid Flowcell Barcode. Please verify and try again.::)
};
if (input.::Flowcell Type:: == ::S1:: && !output.container.name.matches(::[A-Z0-9]{5}DR[A-Z0-9]{2}::)) {
fail(::Invalid Flowcell Barcode. Please verify and try again.::)
};
if (input.::Flowcell Type:: == ::S2:: && !output.container.name.matches(::[A-Z0-9]{5}DM[A-Z0-9]{2}::)) {
fail(::Invalid Flowcell Barcode. Please verify and try again.::)
};
if (input.::Flowcell Type:: == ::S4:: && !output.container.name.matches(::[A-Z0-9]{5}DS[A-Z0-9]{2}::)) {
fail(::Invalid Flowcell Barcode. Please verify and try again.::)
};
```

Generate Sample Sheet & Run Recipe Automation

Automatically triggered when a button on the Record Details screen is selected, this automation:

- Copies the Flowcell Type from the step input to the Run Mode field (hidden):

```
step.::Run Mode:: = input.::Flowcell Type::
```

- Validates the parameters entered on the Record Details screen:
  - Experiment Name can only contain alphanumeric, dash, or underscore characters. Spaces are not permitted.
  - When Workflow Type is No Index, Index Read 1 must be zero. For any other Workflow Type, Index Read 1 must be greater than zero.

```
if(!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) {
fail(::Experiment Name contains prohibited characters. Allowed characters are:
a-z, A-Z, 0-9, -, and _::)
};
if(step.::Workflow Type::== ::No Index::) {
if(step.::Index Read 1::!= 0) {
fail(::Index Read 1 must be 0 if the Workflow Type is No Index.::)
}
} else{
if(step.::Index Read 1::== 0) {
fail(::Index Read 1 must be greater than 0 if the Workflow Type is ::+
step.::Workflow Type::+ ::.::)
}
};
```

  - When Paired End is True, Index Read 1 and Index Read 2 must be greater than zero.
  - When Paired End = False, Index Read 2 must be zero. Reverse Complement Workflow must be false. UMI - Read 2 Length and UMI - Read 2 Start from Cycle must not have a value.

```
if (step.::Paired End::.toBoolean()) {if (step.::Read 1 Cycles:: == 0 ||
step.::Read 2 Cycles:: == 0) {fail(::Read 1 Cycles and Read 2 Cycles must be
greater than 0 if Paired End is True.::);}} else {if (step.::Read 2 Cycles:: !=
0) {fail(::Read 2 Cycles must be 0 if Paired End is False.::);}
if (step.hasValue(::UMI - Read 2 Length::) || step.hasValue(::UMI - Read 2 Start
From Cycle::)) {fail(::UMI - Read 2 Length and UMI - Read 2 Start From Cycle
cannot be defined if Paired End is False.::);}}
```

  - Validates allowed read cycles to be not greater than 151 if the Flowcell Type is not SP.

```
if (input.::Flowcell Type:: != ::SP:: && step.::Read 1 Cycles:: > 151) {
fail(::Read 1 Cycles must not be larger than 151 if it is not SPrime
Flowcell.::);
}
if (input.::Flowcell Type:: != ::SP:: && step.::Read 2 Cycles:: > 151) {
fail(::Read 2 Cycles must not be larger than 151 if it is not SPrime
Flowcell.::);
}
```

- Validates allowed value for UMI - Read 1 Length, UMI - Read 2 Length, UMI - Read 1 From Cycle, UMI - Read 2 From Cycle.

```
if (step.hasValue(::UMI - Read 1 Length::) && !step.hasValue(::UMI - Read 1
Start From Cycle::)) {
fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 1
Length is greater than 0.::);
}
if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 1
Start From Cycle::)) {
fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 1 Start From
Cycle is greater than 0.::);
}
if (step.hasValue(::UMI - Read 2 Length::) && !step.hasValue(::UMI - Read 2
Start From Cycle::)) {
fail(::UMI - Read 2 Start From Cycle must be greater than 0 if UMI - Read 2
Length is greater than 0.::);
}
if (!step.hasValue(::UMI - Read 2 Length::) && step.hasValue(::UMI - Read 2
Start From Cycle::)) {
fail(::UMI - Read 2 Length must be greater than 0 if UMI - Read 2 Start From
Cycle is greater than 0.::);
}
if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 2
Length::)) {
fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 2 Length is
greater than 0.::);
}
if (!step.hasValue(::UMI - Read 1 Start From Cycle::) && step.hasValue(::UMI -
Read 2 Start From Cycle::)) {
fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 2
Start From Cycle is greater than 0.::);
}
```

- Copies the Flowcell Type value from the step inputs to the step outputs:

```
output.::Flowcell Type::= input.::Flowcell Type::;
```

- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol—AUTOMATED - Run (NovaSeq 6000) v2.3.

```
nextStep = ::ADVANCE::
```

- Generates the sample sheet and the run recipe file and attaches them to the step. For details on how the files are generated, and their contents, refer to *Sample Sheet and Run Recipe File Generation* on page 969.

Prepare Files for NovaSeq

Automatically triggered on exit of the Record Details screen, this automation invokes the `copy_attachments_to_network_folder` script, which is included in the `novaseq-remote-extensions.jar` file. This script places the generated sample sheet and run recipe (*json file) into the appropriate folder on the NAS, where the instrument uses them to start the run.

For more information, refer to *Sample Sheet and Run Recipe File Generation* on page 969.

Master Step Fields

The following table provides field configuration details for the fields defined on the Load to Flowcell (NovaSeq 6000 v2.3) step.

Table 230 Load to Flowcell (NovaSeq 6000 v2.3) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Drop-Down Items
BaseSpace Sequence Hub Configuration	Text Dropdown	Required Field	Presets • Not Used • Run Monitoring Only • Run Monitoring and Storage
Experiment Name	Text	Required Field	
Index Read 1	Numeric Dropdown	Required Field Custom Entries	Range = 0–20 Decimal places displayed = 0 Presets • 0 • 6 • 8
Index Read 2	Numeric Dropdown	Required Field Custom Entries	Range = 0–20 Decimal places displayed = 0 Presets • 0 • 6 • 8
Library Tube Barcode	Text	Required Field	

Field Name	Field Type	Options	Additional Options and Drop-Down Items
Paired End	Text Dropdown	Required Field	Presets • True • False
Read 1 Cycles	Numeric Dropdown	Required Field Custom Entries	Range = 1–251 Decimal places displayed = 0 Presets • 251* • 151 • 101 • 51 *The value of 251 is only supported for SP flow cell type. For all other flow cell types, the maximum value is 151.
Read 2 Cycles	Numeric Dropdown	Required Field Custom Entries	Range = 0–251 Decimal places displayed = 0 Presets • 251* • 151 • 101 • 51 *The value of 251 is only supported for SP flow cell type. For all other flow cell types, the maximum value is 151.
Reverse Complement Workflow	Toggle Switch	Required Field	Default • Yes

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Field Name	Field Type	Options	Additional Options and Drop-Down Items
Run Mode (not displayed in user interface)	Text Dropdown	Read Only	Presets • SP • S1 • S2 • S4
Sample Sheet Path	Text	Read Only	
UMI - Read 1 Length	Numeric		Range = 1 Decimal places displayed = 0
UMI - Read 1 Start From Cycle	Numeric		Range = 1 Decimal places displayed = 0
UMI - Read 2 Length	Numeric		Range = 1 Decimal places displayed = 0
UMI - Read 2 Start From Cycle	Numeric		Range = 1 Decimal places displayed = 0
Use Custom Index Read 1 Primer	Toggle Switch		Default • No
Use Custom Read 1 Primer	Toggle Switch		Default • No
Use Custom Read 2 Primer	Toggle Switch		Default • No
Workflow	Text	Read Only	Default • GenerateFASTQ
Workflow Type	Text Dropdown	Required Field	Presets • No Index • Single Index • Dual Index

Global Fields

The following table lists the global fields that are configured to display on the Load to Flowcell (NovaSeq 6000 v2.3) step.

Field Name	Field Type	Options	Additional Options and Drop-Down Items
Configured on Derived Sample			
Flowcell Type	Text Dropdown	Required Field	Presets • S1 • S2 • S4 • SP

User Interaction

1. On the Ice Bucket screen, select the appropriate flow cell type from the Destination Container drop-down list in the Container Options panel.
2. Select Begin Work.
3. On the Placement screen, move the pools as follows.
   a. Drag the pools from the left of the screen over into the Placed Samples area on the right.
   b. Scan or type the barcode of the flow cell into the flow cell field.
   c. Select Record Details.

   On exit of the Placement screen, the Validate Flowcell Barcode automation validates the container barcode (described previously). The fields displayed on the Record Details screen are used to generate the sample sheet and run recipe files. Some of these fields are autopopulated and some must be completed manually.

Table 231 Fields Displayed on Record Details Screen of Load to Flowcell (NovaSeq 6000 v2.3) Step

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.
BaseSpace Sequence Hub Configuration	Presets • Not Used • Run Monitoring Only • Run Monitoring and Storage
Workflow	Presets • GenerateFASTQ

For Research Use Only. Not for use in diagnostic procedures.

Field	Value
Workflow Type	Presets • No Index • Single Index • Dual Index
Index Read 1	Presets • 0 • 6 • 8 • Type a value between 0 and 20
Index Read 2	Presets • 0 • 6 • 8 • Type a value between 0 and 20
Paired End	Presets • True • False
Read 1 Cycles	Presets • 251 • 151 • 101 • 51 • Type a value between 1 and 251 for the SP flow cell type or between 1 and 151 for all other types
Read 2 Cycles	Presets • 251 • 151 • 101 • 51 • Type a value between 0 and 251 for the SP flow cell type or between 0 and 151 for all other types
Use Custom Read 1 Primer	Select if applicable.
Use Custom Read 2 Primer	Select if applicable.
Use Custom Index Read 1 Primer	Select if applicable.

Field	Value
Reverse Complement Workflow	Select Yes (by default) for v1.5 reagents in NovaSeq Control Software v1.7 and onwards. Select No for v1 reagents.
UMI - Read 1 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 1. Leave blank if not applicable.
UMI - Read 2 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 2. Leave blank if not applicable.
UMI - Read 1 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.
UMI - Read 2 Start From Cycle	Enter the cycle number that Read 2 starts from. Leave blank if not applicable.
Sample Sheet Path	Automatically populated after the sample sheet has been generated.
Library Tube Barcode	Scan the library tube barcode.

4. On the Record Details screen, select Generate Sample Sheet & Run Recipe.
   This selection triggers the automation script, which does the following actions:
   - Generates the sample sheet and JSON files and attaches them to the placeholders in the Files area of the Record Details screen.
   - Populates the Sample Sheet Path field with the path to the sample sheet file.
5. Select Next Steps to trigger the Prepare Files for NovaSeq automation, which copies the sample sheet and run recipe files to the location specified during installation.
   On the Assign Next Steps screen, the Next Step for all samples is prepopulated with Mark protocol as complete.
6. Select Finish Step.
   In Lab View, the pool of samples is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) step.

At this point in the workflow, the user interaction ends. The integration automatically starts the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) step and data from the run is parsed back into Clarity LIMS.

Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3)

This final protocol contains one fully automated step, AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3).

Step 1: AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3)

Step input: Library tube from the NovaSeq Standard protocol or flow cell from the NovaSeq Xp protocol.

Step output: Result file and measurement.

> Do not add samples to the Ice Bucket or start the step manually. This is a fully automated step. The sequencing service does not update samples correctly if they have been manually started.

In this step, pooled samples are sequenced on the NovaSeq instrument. The run data network folder is monitored by the sequencing service and automation worker to determine when the sequencing run begins and ends.

1. When a run is initiated on the NovaSeq instrument, the NovaSeq Control Software (NVCS) looks for the sample sheet and run recipe (*.json file) on the shared network drive.
   - The NVCS copies the sample sheet to the run directory.
   - The NVCS uses the run recipe to start the sequencing run.
2. When the run starts:
   - The instrument software creates a new run folder (named < libraryID >) on the shared network drive and copies the following files into that folder:
     - RunParameters.xml
     - RunInfo.xml
     - LIMS/ <libraryID>.json file (run recipe)
   - The Real-Time Analysis v3 (RTA3) software creates the InterOp folder.
3. The sequencing service detects the presence of the RunParameters, RunInfo, and JSON files and starts the AUTOMATED- NovaSeq Run (NovaSeq 6000 v2.3) step in Clarity LIMS.
4. As the run progresses, the InterOp data files are filled in.
5. At the end of the run, the NovaSeq:
   - Copies the run data files to the InterOp folder.
   - Creates the `CopyComplete.txt` file in the run folder. This step indicates to the sequencing service that the run has completed and the data files are ready.
6. The sequencing service triggers the Read InterOp Metrics automation. This automation reads the files, records the parsed metrics into Clarity LIMS, and finally completes the step in Clarity LIMS.

Parse Run Parameters and Generate Link to Run Folder Automation

Automatically triggered on entry to the Record Details screen, this automation does the following actions:

- Parses the `RunParameters.xml` file attached on the Step Setup screen.
- Populates the master and global step fields with extracted values.

- If there is an instrument in Clarity LIMS that matches the instrument name in the file (InstrumentName field), the script associates this instrument with the run.
- Generates and attaches a link to the run folder using the run output folder parsed from `RunParameters.xml`.

The following rules and constraints are present:

- If there is no InstrumentName value in the file, the script continues, but it logs a warning.
- If there is an InstrumentName value in the file and multiple instruments with the same name exist in Clarity LIMS, the script fails and logs an error.
- If the `RunParameters.xml` file is not attached to the step during step setup, the script fails.
- Every `RunParameters.xml` field that has an equivalent step UDF/custom field in Clarity LIMS is required—except for InstrumentName. If a field is not present in the `RunParameters.xml` file, Clarity LIMS logs an error to this effect.

Master Step Fields

The fields defined on the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) step are listed in the Clarity LIMS Configuration area (Custom Fields > Master Step Fields tab).

The following table shows how the parsed `RunParameters.xml` fields map to step fields configured on the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) step in Clarity LIMS.

Table 232 Fields Parsed by parse_novaseq_run_parameters script

RunParameters.xml Field	Field Name
FlowCellSerialBarcode	Flow Cell ID
FlowCellPartNumber	Flow Cell Part Number
FlowCellLotNumber	Flow Cell Lot Number
FlowCellExpirationdate	Flow Cell Expiration Date
FlowCellMode	Flow Cell Mode
RunId	Run ID
ClusterSerialBarcode	PE Serial Barcode
ClusterPartNumber	PE Part Number
ClusterLotNumber	PE Lot Number
ClusterExpirationdate	PE Expiration Date
ClusterCycleKit	PE Cycle Kit
SbsSerialBarcode	SBS Serial Barcode

RunParameters.xml Field	Field Name
SbsPartNumber	SBS Part Number
SbsLotNumber	SBS Lot Number
SbsExpirationdate	SBS Expiration Date
SbsCycleKit	SBS Cycle Kit
BufferSerialBarcode	Buffer Serial Barcode
BufferPartNumber	Buffer Part Number
BufferLotNumber	Buffer Lot Number
BufferExpirationdate	Buffer Expiration
OutputRunFolder	Output Folder
WorkflowType	Loading Workflow Type

Read InterOp Metrics Automation

Automatically triggered when the sequencing service detects the `CopyComplete.txt` file has been created, this automation parses the InterOp data generated by the run.

- Read summary metrics are recorded on the library pool. These values are aggregated across all lanes. Some values—for example Yield PF (Gb)—are summed, while others are averaged.
- Per lane metrics are recorded on the measurement outputs.

The `read_interop_metrics` script loads data from the InterOp files and records summary metrics into derived sample UDFs/custom fields in Clarity LIMS. If any field is not configured or cannot be written to, the script fails.

Global Fields

The global fields that are configured to support the parsing of key metrics back into Clarity LIMS are as follows.

- Yield PF (Gb) R1
- Yield PF (Gb) R2
- Reads PF (M) R1
- Reads PF (M) R2
- Cluster Density (K/mm^2) R1
- Cluster Density (K/mm^2) R2
- %PF R1
- %PF R2

- % Bases >=Q30 R1
- % Bases >=Q30 R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- % Aligned R1
- % Aligned R
- % Error Rate R1
- % Error Rate R2

Set Next Steps Automation

Automatically triggered on exit of the Record Details screen, the following automation sets the next step to ADVANCE and the samples complete the protocol:

```
nextStep = ::ADVANCE::
```

Update Lane Number Automation

Automatically triggered on exit of the Step Setup screen, this automation updates the lane number so that Read InterOp Metrics automation script can populate lane metric to the correct lane.

> Do not disable Update Lane Number automation as it causes run metric parsing to work improperly.

Components Installed

The following sections describe the various components that are installed by default as part of this integration. These components include files, database properties, reagent categories/label groups, reagent kits, and containers. Information on installed workflows, protocols, steps, and automation points is provided in the previous *Workflows, Protocols, and Steps* section.

The Illumina NovaSeq 6000 Integration v2.5 Package is distributed as two RPM packages:

- BaseSpaceLIMS-novaseq-extensions
- BaseSpaceLIMS-novaseq-sequencing-service

The RPM packages install the components listed in the following table.

The NovaSeq 6000 Integration requires installation of NGS Extensions Package v5.23 or later.

- If the NGS Extensions Package is not already installed, or if a version earlier than v5.23 is installed, the latest version is installed by default with the NovaSeq integration. For details, refer to *NovaSeq 6000 Integration v2.5 Release Notes* on page 1002.
- If NGS Extensions Package v5.23 is already installed, upgrade is not forced or required.
- Secret Util Package is required for the NGS Extensions Package v5.23.
- If the Secret Util Package is not already installed, then Secret Util package is installed along with the installation of NGS Extension Package v5.23.
- If the Secret Util Package is already installed, upgrade is not forced or required.

Table 233 Illumina NovaSeq 6000 Integration v2.5 RPM Components

Files Installed	Location and Description
`configure_extensions_novaseq.sh`	`/opt/gls/clarity/config/` Script that imports Clarity LIMS configuration into the application database and updates property values.
`novaseq-extensions.jar`	`/opt/gls/clarity/extensions/novaseq` Jar file containing API-based Clarity LIMS extensions used throughout the protocols.
`novaseq-sequencing.jar`	`/opt/gls/clarity/extensions/novaseq/SequencingService` Sequencing service jar file that captures the run results.
`novaseq-remote-extensions.jar`	`/opt/gls/clarity/extensions/novaseq` Jar file containing scripts that require access to the run folders on the NAS.
InterOp libraries	`/opt/gls/clarity/extensions/novaseq/lib` Illumina shared library for parsing InterOp data files.

`NovaSeq_Standard_Bulk_Pool1.csv`
`NovaSeq_Standard_Bulk_Pool2.csv`
`NovaSeq_Standard_Bulk_Pool3.csv`
`NovaSeq_Xp_Bulk_Pool.csv`
`NovaSeq_Xp_Bulk_Pool2.csv`
`NovaSeq_Xp_Working_Pool.csv`
`NovaSeq_Xp_Working_Pool2.csv`
	`/opt/gls/clarity/extensions/novaseq/Templates`
	Template files used for file generation throughout the protocols.

Database Properties

The following table lists the database properties installed with the Illumina NovaSeq 6000 Integration v2.5 Package.

Table 234 Database Properties Installed

Property	Description
novaseq.runSetupFolder	The directory where the run recipe files are written.
novaseq.sampleSheetPathPrefixSearch.SUFFIX	The directory where the instrument control software or analysis software looks for sample sheets, typically a Windows path to a network drive.
novaseq.sampleSheetPathPrefixReplace.SUFFIX	The directory where the sample sheets are written.
novaseq.sampleSheetPathPrefixSearchReplaceSuffixes[1]	Search and replace entries for transforming Windows to Linux network paths. This property must be set to the single SUFFIX used in the two previous properties.
novaseq.seqservice.netPathPrefixSearch.SUFFIX	The search prefix used to map the OutputRunFolder field in `RunParameters.xml` to the corresponding Linux directory.
novaseq.seqservice.netPathPrefixReplace.SUFFIX	The directory where the sequencing service looks for run folders.
novaseq.seqservice.netPathPrefixSearchReplaceSuffixes[1]	Search and replace entries for transforming Windows to Linux network paths. This property must be set to the single SUFFIX used in the two properties above.

[1]This version of the integration only supports a single active search-replace pair of each type:sampleSheetPathPrefixSearchReplaceSuffixes and netPathPrefixSearchReplaceSuffixesmust each contain a single number, rather than a comma-separated list of numbers.

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Label Groups, Reagent Kits, and Containers

The NovaSeq 6000 Integration v2.5 Package installs the following label groups, reagent kits, and container types.

Label Groups

- TruSeq HT Adapters v2 (D7-D5)

Reagent Kits

- DPX1
- DPX2
- DPX3
- Resuspension Buffer (RSB)
- NaOH
- Tris-HCl

Container Types

- Library Tube
- S1
- S2
- S4
- SP

Instrument Integration

The following are instructions for configuring the NovaSeq instrument for the Clarity LIMS integration:

1. Launch NovaSeq Control Software.
2. On the home screen, select the menu (top left) and select Settings.
3. Select File-Based and enter the network folder location that NovaSeq Control Software retrieves the JSON recipe from.
4. Enter the network output folder location.
5. Select Save.

For more information, refer to the *NovaSeq 6000 Sequencing System Guide* at support.illumina.com.

Routing Script Requirements

The requirements for the routing script functionality are as follows.

- On the steps that use the routing script—Define Run Format (NovaSeq 6000 v2.3) and Dilute and Denature (NovaSeq 6000 v2.3)—the Next Step for all samples must be set to Remove from workflow. This value is set by a script. Do not change this value in the Assign Next Steps screen.

- In the protocol configuration screen, the following setting is required and must not be changed.
  - In the Next Steps section for the last step in the protocol, the method of assigning the next step must be set to Automatic.

Sample Sheet and Run Recipe File Generation

Sample sheet and run recipe file generation occurs on the step before the run—Dilute and Denature (NovaSeq 6000 v2.3) in the NovaSeq Standard protocol or Load to Flowcell (NovaSeq 6000 v2.3) in the NovaSeq Xp protocol. This step is where samples are placed on the library tube or flow cell that is loaded in the NovaSeq instrument.

In the default configuration, the Generate Sample Sheet & Run Recipe automation generates the following:

- One CSV format sample sheet file for use bcl2fastq v2.20 downstream analysis
- One JSON format run recipe file

The Prepare Files for NovaSeq automation places the generated files on the NAS where the instrument uses them to start the run.

How It Works

- The Prepare Files for NovaSeq automation command line invokes the `copy_attachments_to_network_folder` script `in novaseq-remote-extensions.jar`. The script can be used on a remote AI node/automation worker, in case the Clarity LIMS server does not have direct access to the NAS.
- The output folder for the run recipe file is found in the `novaseq.runSetupFolder` property.
- The output folder for the sample sheet is determined by using the `novaseq.sampleSheetPathPrefixReplace.<suffix>` property (not a comma-separated list).

Sample Sheet Contents

The contents of the sample sheet are controlled by the following fields configured on the Dilute and Denature (NovaSeq 6000 v2.3) and Load to Flowcell (NovaSeq 6000 v2.3) steps:

- Experiment Name
- Read 1 Cycles
- Read 2 Cycles
- Workflow
- Reverse Complement Workflow
- Read1UMILength
- Read2UMILength

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- Read1StartFromCycle
- Read2StartFromCycle

Refer to the *Bcl2fastq2 Sample Sheet Generation* section of the *Illumina Instrument Sample Sheets (NGS v5.17 & later)* on page 2542 document for detailed information on the following:

- Sample sheet generation script parameters and usage
- Sample sheet data and configuration options
- Enabling unique FASTQ file names per sequencing run

Run Recipe Contents

The contents of the run recipe are controlled by the following fields. These fields are configured on the Dilute and Denature (NovaSeq 6000 v2.3) and Load to Flowcell (NovaSeq 6000 v2.3) steps.

- Experiment Name
- Run Mode
- Workflow Type
- Sample Loading Type (populated based on Workflow field)
- Paired End
- Read 1
- Read 2
- Index Read 1
- Index Read 2
- Sample Sheet Path
- BaseSpace Configuration
- Use Custom Read 1 Primer
- Use Custom Read 2 Primer
- Use Custom Index Read 1 Primer

The following fields are also included in the run recipe:

- Library Tube Barcode (librarytube_ID in JSON file)
  - NovaSeq Standard workflow—Library tube barcode is derived from the container name entered or scanned on the Placement screen.
  - NovaSeq Xp workflow—Library tube barcode is derived from the Library Tube Barcode field in the Load To Flowcell (NovaSeq 6000 v2.3) step.
- Flowcell Barcode (flowcell_ID in JSON file)
  - NovaSeq Standard workflow—Library tube barcode is not output to JSON recipe file.

  - NovaSeq Xp workflow—Flow cell barcode is derived from the container name entered or scanned on the Placement screen.
- Rehyb—preset to False.
- Output Folder (output_folder in JSON file)—Derived from the system property novaseq.seqservice.netPathPrefixSearch.
- Attachment—Derived from the system property novaseq.sampleSheetPathPrefixSearch with the name of the sample sheet added to the end of the path.
- Use BaseSpace (use_basespace in JSON file)—Set to False if the BaseSpace Sequence Hub Configuration step field value is Not Used, and true otherwise.

Rules and Constraints

- The sample sheet and run recipe files are mandatory inputs for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) step. For the automated run to start successfully, select the Generate Sample Sheet & Run Recipe button to generate these files.
- The output folders must exist on the system where the script runs (can be a remote AI node/automation worker).
- The output folders must be writable by the glsai user.

Container Types

The integration supports the following container types:

- Library tube with barcode provided in the following format:
  - [A-Z]{2}[0-9]{7}-[A-Z]{3}
  - Example—NV1234567-LIB
- Flow cell with barcode provided in one of the following formats:
  - For SP and S1—[A-Z0-9]{5}DR[A-Z0-9]{2}
  - For S2—[A-Z0-9]{5}DM[A-Z0-9]{2}
  - For S4—[A-Z0-9]{5}DS[A-Z0-9]{2}
  - Example S2 flow cell barcode—H1234DMXX

Rules and Constraints

- The workflow configuration contains several validation checks. To make sure that the calculations work properly, it is important that you do not disable any of this validation logic. The validation checks the following information:
  - Which samples, and how many, can enter each step together.
  - Which samples, and how many, can be pooled together.
- Reagent labels (indexes) must be unique.

- The library tube ID must be unique. There should not be multiple library tube containers in the system with the same name.
- Only controls are permitted as unindexed samples. All other unindexed samples and pools are not permitted.
- Refer to the *Bcl2fastq2 Sample Sheet Generation* section of the *Illumina Instrument Sample Sheets (NGS v5.17 & later)* on page 2542 document for detailed information on the following:
  - Sample sheet generation script parameters and usage
  - Sample sheet data and configuration options
  - Enabling unique FASTQ file names per sequencing run
- Do not manually start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) step. This step is fully automated. If the samples have been manually started, the sequencing service does not update them correctly.
- The output folder must be readable by the Clarity LIMS glsjboss account on the server.

NovaSeq 6000 Integration v2.5 Validation and Troubleshooting

This guide explains how to validate the installation of the Illumina NovaSeq 6000 Integration Package v2.5. The validation process involves the following actions:

- Running samples through the NovaSeq Validation Library Prep (NovaSeq 6000 v2.3) workflow. The workflow contains a single-step protocol that models the library prep required to produce normalized libraries. At the end of the step, the normalized libraries are automatically advanced to the NovaSeq 6000 v2.3 workflow.
- Running normalized libraries through the NovaSeq 6000 v2.3 workflow. This process validates the following details:
  - Successful routing of samples from the Run Format (NovaSeq 6000 v2.3) step to the NovaSeq Standard (NovaSeq 6000 v2.3) or NovaSeq Xp (NovaSeq 6000 v2.3) step.
  - Automated generation of a sample sheet for use with bcl2fastq2 v2.20 analysis software.
  - Automated generation of a run recipe file (JSON file) with the library tube or flow cell barcode as the name (eg, NV1234567-LIB.json or H1234DRXX.json). This file is automatically uploaded to the sequencing instrument and used to set up and initiate the run.
  - Automated tracking of the NovaSeq sequencing run and parsing of run statistics (per run per lane) into Clarity LIMS.

Before executing the validation steps, make sure that you have installed the Illumina NovaSeq 6000 Integration Package v2.5 and have imported the default Clarity LIMS configuration.

Activate Workflow, Create Project, Add and Assign Samples

The following steps set up Clarity LIMS in preparation for running samples through the NovaSeq Validation Library Prep (NovaSeq 6000 v2.3) and NovaSeq 6000 v2.3 workflows.

1. In the Clarity LIMS Configuration area, activate the NovaSeq Validation Library Prep (NovaSeq 6000 v2.3) and NovaSeq 6000 v2.3 workflows.
2. On the Projects and Samples screen, create a project and add samples to it.
3. Assign the samples to the NovaSeq Validation Library Prep (NovaSeq 6000 v2.3) workflow.

Library Prep Protocol: NovaSeq Validation Library Prep (NovaSeq 6000 v2.3)

This single-step protocol models the library prep required to produce normalized libraries that are ready for the NovaSeq 6000 v2.3 workflow.

Run NovaSeq Validation Library Prep (NovaSeq 6000 v2.3)

1. In Lab View, locate the NovaSeq Validation Library Prep (NovaSeq 6000 v2.3) protocol. The samples are queued for the NovaSeq Validation Library Prep (NovaSeq 6000 v2.3) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Placement screen, scan or enter the barcode of the destination container into the Container field of the Placed Samples area.
   a. Select the samples from the Samples to be Placed area on the left, then drag them over to the container wells on the right.
   b. Select Add Labels.
5. On the Add Labels screen, select labels from the Labels to Add list and drag them to the container on the right to assign them to placed samples.
   The TruSeq HT Adapters v2 (D7-D5) label group is the only label group configured in the out-of-the-box integration and is the default in this step.
   a. Select Record Details.
6. On the Record Details screen in the Sample Details table, enter values for Normalized Molarity. Type values individually or use the copy/paste functionality as follows.
   a. Select the Copy button to copy the sample information from Clarity LIMS to the clipboard.
   b. Open a blank Excel document and paste the contents of the clipboard into the spreadsheet.
   c. In the spreadsheet, enter the Normalized Molarity values. Select the entire spreadsheet, including the headers (Ctrl + A), and copy (Ctrl + C) the information to the clipboard.
   d. In Clarity LIMS, select the Paste button. Paste the contents of the clipboard (Ctrl + V) into the Update Table window, then select Update to paste into the Sample Details table.

   The Sequencer Type field indicates the instrument to be used for the sequencing run. In this integration, the value of this field is set to NovaSeq automatically.

7. Select Next Steps.
   This selection triggers the Next Steps automation, which sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step successfully.
8. On the Assign Next Steps screen in the Samples table, the Next Step for all samples is prepopulated with Remove from workflow.

   Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.

9. Select Finish Step.
   On exit from the step, the Routing Script automation is triggered. This automation assigns samples to the first step of the NovaSeq 6000 v2.3 workflow—Define Run Format (NovaSeq 6000 v2.3). This is the only step in Protocol 1: Run Format (NovaSeq 6000 v2.3).

Protocol 1: Run Format (NovaSeq 6000 v2.3)

This protocol includes the Define Run Format (NovaSeq 6000 v2.3) step. The step allows for the assignment of per sample values for Loading Workflow Type, Normalized Molarity, Flowcell Type, and Final Loading Concentration (pM). At the end of the step, samples are routed to the NovaSeq Standard or NovaSeq Xp protocol, according to the selected Loading Workflow Type.

Step 1: Run Define Run Format (NovaSeq 6000 v2.3)

1. In Lab View, locate the Run Format (NovaSeq 6000 v2.3) protocol. The samples are queued for the Define Run Format (NovaSeq 6000 v2.3) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Record Details screen, specify the Sample Details.
   - Loading Workflow Type—Select NovaSeq Standard or NovaSeq Xp from the drop-down list.
   - Normalized Molarity—Enter values or use values copied from the previous step.
5. Populate the following fields:

Values can vary across samples.

   - Flowcell Type—Select SP, S1, S2, or S4.
   - Final Loading Concentration (pM)—Select from the two preset options—225 (for PCR-free workflows) or 400 (for Nano workflows)—or enter a different value.
6. Select Next Steps to trigger the Set Next Steps automation, which does the following actions:
   - Sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step successfully.
   - Calculates the Minimum Molarity.

- Checks Normalized Molarity value. For samples with no Normalized Molarity value (eg, an empty value, not including 0), the automation generates an error message stating that the field cannot be empty.
- Compares the Normalized Molarity value of each sample with the Minimum Molarity value.

7. On the Assign Next Steps screen, review the Sample Details table and make sure that the Next Step for all samples is prepopulated with Remove from workflow.
   Next Step must be set to Remove from workflow, regardless of the Loading Workflow Type.

   If the Next Step value is not set to Remove from workflow, the routing script automation cannot advance to the next step.

8. Review the Warning field entries for the samples. If the field indicates that the Normalized Molarity is too low, continue with one of the following options:
   - Return to the Record Details screen and adjust the Normalized Molarity value so that it equals or exceeds the Minimum Molarity value. Set the Loading Workflow Type to NovaSeq Standard or NovaSeq Xp, as applicable.
   - Alternatively, complete the protocol without correcting the Normalized Molarity value. In this case, the samples in question are removed from the Clarity LIMS workflow.
9. Select Finish Step.
   The Routing Script automation is triggered if:
   - Samples with a Loading Workflow Type of Remove from workflow (ie, where Normalized Molarity value is lower than the Minimum Molarity) are removed from the Clarity LIMS workflow.
   - Samples with a Loading Workflow Type of NovaSeq Standard are routed to the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.3) step. This step is the first of two steps in Protocol 2: NovaSeq Standard (NovaSeq 6000 v2.3).
   - Samples with a Loading Workflow Type of NovaSeq Xp are routed to the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.3) step. This step is the first of three steps in Protocol 3: NovaSeq Xp (NovaSeq 6000 v2.3).

Protocol 2: NovaSeq Standard (NovaSeq 6000 v2.3)

In this protocol, samples are pooled and added to the library tube in preparation for the NovaSeq run. The protocol contains two steps:

1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.3)
2. Dilute and Denature (NovaSeq 6000 v2.3)

Step 1: Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.3)

1. In Lab View, locate the NovaSeq Standard (NovaSeq 6000 v2.3) protocol. The samples are queued for the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.3) step.

2. On the Queue screen, add samples of the same flow cell type to the Ice Bucket and select Begin Work.
3. On the Pooling screen, create a pool by dragging samples into the Pool Creator. Only one pool must be created.
   a. Type a name for the pool or accept the default name (Pool #1).
   b. Select Record Details.
4. On exit from the Pooling screen, the Validate Inputs Flowcell Type and Single Pool is triggered. The automation checks the following information:
   - All samples in the pool have been assigned the same Flowcell Type.
   - Only one pool has been created.
5. On the Record Details screen in the Step Details area, complete the fields as follows.
   a. Enter the value for the Number of Flowcells to Sequence field.
   This value is used in volume calculations to make sure that the volumes are sufficient for the number of times the pool is sequenced.
   b. Enter the value for the Minimum Per Sample Volume (µl) field.
   The value in this field is used to calculate how much of each sample is included in the pool. The field is prepopulated with the configured default value (5 µl), but the value can be edited if necessary.
   c. If necessary, enter the value for the % PhiX (2.5 nM) Spike-In field.
   The value in this field is used to calculate the volume of PhiX v3 control to be included in the pool for the given percentage of spike-in. The field is optional and can be edited.

   Assuming the default Minimum Per Sample Volume (µl) value of 5, for a given batch:
   - If the smallest Per Sample Volume (µl) value is less than 5, the LIMS automatically assigns a value of 5 to the sample's Adjusted Per Sample Volume (µl) field.
   - Clarity LIMS then adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.
6. In the Sample Details table, select the pool icon to view details on the pool composition.
7. Select Calculate Volumes to trigger the Calculate Volumes automation, which performs volume calculations based on the selected Flowcell Type:
   - Calculates Bulk Pool Volume (µl) value.
   - Calculates the Per Sample Volume (µl) value to be added to the pool.
   - Calculates PhiX Volume (µl) value if % PhiX (2.5 nM) Spike-In has value.
   - Calculates the Total Sample Volume (µl) value.
   - If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) value.

- Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
- Populates the Volume of Pool to Denature (µl), NaOH Volume (µl) and Tris-HCl Volume (µl) columns of the Sample Details table. (Values are set by a script and are not editable.)
- The automation also generates the Calculation File (*csv) and attaches it to the step. This file contains volume information about the pool and the individual samples it contains. Select the file to download it, then open it in Excel.

8. Select Next Steps to trigger the Set Next Steps automation.
   This automation sets the next step for samples to ADVANCE, advancing them to the Dilute and Denature (NovaSeq 6000 v2.3) step in the protocol.
9. Select Finish Step.

At the end of this step, the pool of samples automatically advances to the Dilute and Denature (NovaSeq 6000 v2.3) step.

Step 2: Dilute and Denature (NovaSeq 6000 v2.3)

1. In Lab View, locate the NovaSeq Standard (NovaSeq 6000 v2.3) protocol. The pool of samples is queued for the Dilute and Denature (NovaSeq 6000 v2.3) step.
2. Add the samples to the Ice Bucket and select Begin Work.
   At the beginning of the step, the Validate Single Input automation is triggered. This automation checks that there is only one container input to the step.
3. On the Placement screen, drag the pool into the library tube in the Placed Samples area.
   a. Scan or type the barcode of the library tube into the Library Tube field.
   b. Select Record Details.
   On exit of the Placement screen, the Validate Library Tube Barcode automation checks that the library tube barcode conforms to the barcode mask [A-Z]{2}[0-9]{7}-[A-Z]{3}. If not, an error message displays.
4. On the Record Details screen, the Reagent Lot Tracking section tracks the NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. To add and activate reagent lots, complete the following steps:
   a. In a new browser tab, open an additional instance of Clarity LIMS.
   b. Navigate to the Consumables configuration screen.
   c. Select the NaOH reagent kit and select New Lot.
   d. Set the Status of Reagent Lot switch to Active.
   e. Enter the lot details.
   f. Select Save.
   g. Repeat this process to add and activate the Resuspension Buffer and Tris-HCl reagent lots.
   h. Close this browser tab and return to your original working tab. Refresh the page.

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5. In the Reagent Lot Tracking section, select from the active lots displayed in each drop-down list. The fields displayed in the Step Details section are used to generate the sample sheet and run recipe files. Some of these fields are autopopulated and some must be completed manually. Refer to the following table for details.

Table 235 Fields Displayed on Record Details Screen of Dilute and Denature (NovaSeq 6000 v2.3) Step

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. No spaces.
BaseSpace Sequence Hub Configuration	Presets • Not Used • Run Monitoring Only • Run Monitoring and Storage
Workflow	Presets • GenerateFASTQ
Workflow Type	Presets • No Index • Single Index • Dual Index
Index Read 1	Presets • 0 • 6 • 8 • type a value between 0 and 20
Index Read 2	Presets • 0 • 6 • 8 • type a value between 0 and 20
Paired End	Presets • True • False

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Field	Value
Read 1 Cycles	Presets • 251 • 151 • 101 • 51 • type a value between 1 and 251 for the SP flow cell type or between 1 and 151 for all other types
Read 2 Cycles	Presets • 251 • 151 • 101 • 51 • type a value between 0 and 251 for the SP flow cell type or between 0 and 151 for all other types
Use Custom Read 1 Primer	Select if applicable.
Use Custom Read 2 Primer	Select if applicable.
Use Custom Index Read 1 Primer	Select if applicable.
Reverse Complement Workflow	Select Yes (by default) for v1.5 reagents in NovaSeq Control Software v1.7 and onwards. Select No for v1 reagents.
UMI - Read 1 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 1. Leave blank if not applicable.
UMI - Read 2 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 2. Leave blank if not applicable.
UMI - Read 1 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.
UMI - Read 2 Start From Cycle	Enter the cycle number that Read 2 starts from. Leave blank if not applicable.
Sample Sheet Path	Automatically populated after the sample sheet has been generated.

6. On the Record Details screen, select Generate Sample Sheet & Run Recipe. This selection triggers the automation script: The sample sheet and JSON files are generated and attached to the placeholders in the Files area of the Record Details screen.
   The Sample Sheet Path field is populated with the path to the sample sheet file.

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7. Select Next Steps. This selection triggers the Prepare Files for NovaSeq automation, which does the following:
   - Copies the sample sheet and run recipe files to the location specified during installation. The NovaSeq instrument software uses these files to set up the run.
   - Sets the value of the next step to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step successfully.
8. On the Assign Next Steps screen, the Next Step for samples is prepopulated with Remove from workflow.
   Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.
9. Select Finish Step.
   On exit from the step, the Routing Script automation is triggered and samples are routed to Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3).
   In Lab View, the pool of samples is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) step.

Do not add samples to the Ice Bucket or start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) step. The integration does this action automatically.

Protocol 3: NovaSeq Xp (NovaSeq 6000 v2.3)

In this protocol, samples are pooled and added to lanes on the NovaSeq flow cell. The flow cell type is determined by the option selected in the Define Run Format (NovaSeq 6000 v2.3) step.

The protocol contains three steps:

1. Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.3)
2. Dilute, Denature & ExAmp (NovaSeq 6000 v2.3)
3. Load to Flowcell (NovaSeq 6000 v2.3)

Step 1: Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.3)

1. In Lab View, locate the NovaSeq Xp (NovaSeq 6000 v2.3) protocol. The samples are queued for the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.3) step.
2. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucket and select Begin Work.
3. On the Pooling screen, create a pool by dragging samples into the Pool Creator. Only one pool must be created.
   a. Type a name for the pool or accept the default name Pool #1.
   b. Select Record Details.
   On exit from the Pooling screen, the Validate Inputs Flowcell Type and Single Pool is triggered. The automation checks the following details:

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- All samples in the pool have been assigned the same Flowcell Type.
- Only one pool has been created.

4. On the Record Details screen in the Step Details area, complete the fields as follows.
   a. Enter the value for the Number of Lanes to Sequence field.
   This value is used in volume calculations to make sure that the volumes are sufficient for the number of times the pool is sequenced.
   b. Enter the value for the Minimum Per Sample Volume (µl) field.
   The value in this field is used to calculate how much of each sample is included in the pool. The field is prepopulated with the configured default value (5 µl), but the value can be edited if necessary.
   c. If necessary, enter the value for the % PhiX (0.25 nM) Spike-In field.
   The value in this field is used to calculate the volume of PhiX v3 control to be included in the pool for the given percentage of spike-in. The field is optional and can be edited.

   Assuming the default Minimum Per Sample Volume (µl) value of 5, for a given batch:
   - If the smallest Per Sample Volume (µl) value is less than 5, the LIMS automatically assigns a value of 5 to the sample's Adjusted Per Sample Volume (µl) field.
   - Clarity LIMS then adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

5. In the Sample Details table, select the pool icon to view details on the pool composition.
6. Select Calculate Volumes to trigger the Calculate Volumes automation, which performs volume calculations based on the selected Flowcell Type:
   - Calculates Bulk Pool Volume (µl) value.
   - Calculates the Per Sample Volume (µl) value to be added to the pool.
   - Calculates PhiX Volume (µl) value if % PhiX (2.5 nM) Spike-In has value.
   - Calculates the Total Sample Volume (µl) value.
   - If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) value.
   - Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
   - The automation also generates the Calculation File (*csv) and attaches it to the step. This file contains volume information about the samples and RSB buffer to add to the pool. Select the file to download it, then open it in Excel.
7. Select Next Steps to trigger the Set Next Steps automation.
   This automation sets the next step for samples to ADVANCE, advancing them to the Dilute, Denature & ExAmp (NovaSeq 6000 v2.3) step in the protocol.
8. Select Finish Step.

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At the end of this step, the pool of samples automatically advances to the Dilute, Denature & ExAmp (NovaSeq 6000 v2.3) step.

Step 2: Dilute, Denature & ExAmp (NovaSeq 6000 v2.3)

1. In Lab View, locate the NovaSeq Xp (NovaSeq 6000 v2.3) protocol. The pool of samples is queued for the Dilute, Denature & ExAmp (NovaSeq 6000 v2.3) step. Add the pool to the Ice Bucket.
2. On the Ice Bucket screen, set the number of derivatives to create (they are placed into the flow cell lanes) and select Begin Work.
   The Validate Inputs Flowcell Type automation checks that there is only one container input to the step.
3. On entry to the Record Details screen, the Calculate Volumes automation is triggered.
   This automation sets the following values based on the selected Flowcell Type:
   - NaOH Volume (µl)
   - Tris-HCl Volume (µl)
   - DPX1 Volume (µl)
   - DPX2 Volume (µl)
   - DPX3 Volume (µl)
   - Mastermix per Lane (µl)
   - The automation also generates the Calculation File (*csv) and attaches it to the step. This file contains information about the DPX Mastermix volume and the volume of Mastermix, NaOH, and Tris-HCl to add per working pool.
4. On the Record Details screen, the Reagent Lot Tracking section tracks the DPX1, DPX2, DPX3, NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. To add and activate reagent lots, complete the following steps:
   a. In a new browser tab, open an additional instance of Clarity LIMS.
   b. Navigate to the Consumables configuration screen.
   c. Select one of the reagent kits listed and select New Lot.
   d. Set the Status of Reagent Lot switch to Active.
   e. Enter the lot details.
   f. Select Save.
   g. Repeat this process to add and activate the remaining reagent lots.
   h. Close this browser tab and return to your original working tab. Refresh the page.
5. On the Record Details screen in the Reagent Lot Tracking section, select from the active lots displayed in each drop-down list.
   In the Step Details area, the DPX1, DPX2, and DPX3 reagent volume values are already populated. These values are set by a script and are not editable while running the step.

6. In the Sample Details table, select the pool icon to view details on the working pool composition.

> The BP Aliquot, Mastermix per lane, NaOH, and Tris-HCl volume values for each working pool are already populated. These values are set by a script and are not editable while running the step.
>
> The working pool number is appended to the bulk pool name. This feature allows you to identify which working pools are derived from the same bulk pool quickly.

7. [Optional] Select the Calculation File (CSV) in the Files area to view details on the following volumes to add per working pool:
   - DPX Mastermix
   - Mastermix
   - NaOH
   - Tris-HCl
8. Select Next Steps.
   On the Assign Next Steps screen, the next step is already set to Load to Flowcell (NovaSeq 6000 v2.3).
9. Select Finish Step.

Step 3: Run Load to Flowcell (NovaSeq 6000 v2.3)

1. On the Ice Bucket screen in the Container Options panel, select the appropriate flow cell type from the Destination Container drop-down list and select Begin Work.
2. On the Placement screen, drag the pools from the left of the screen over into the Placed Samples area on the right.
   a. Scan or type the barcode of the flow cell into the Flow Cell field.
   b. Select Record Details.
   On exit of the Placement screen, the Validate Flowcell Barcode automation validates the container barcode.

Flowcell Type	Barcode Mask
SP	[A-Z0-9]{5}DR[A-Z0-9]{2}
S1	[A-Z0-9]{5}DR[A-Z0-9]{2}
S2	[A-Z0-9]{5}DM[A-Z0-9]{2}
S4	[A-Z0-9]{5}DS[A-Z0-9]{2}

3. The fields displayed in the Step Details section are used to generate the sample sheet and run recipe files. Some of these fields are autopopulated and some must be completed manually. See the following table for details.

Table 236 Fields Displayed on Record Details Screen of Load to Flowcell (NovaSeq 6000 v2.3) Step

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. No spaces.
BaseSpace Sequence Hub Configuration	Presets • Not Used • Run Monitoring Only • Run Monitoring and Storage
Workflow	Presets • GenerateFASTQ
Workflow Type	Presets • No Index • Single Index • Dual Index
Index Read 1	Presets • 0 • 6 • 8 • type a value between 0 and 20
Index Read 2	Presets • 0 • 6 • 8 • type a value between 0 and 20
Paired End	Presets • True • False
Read 1 Cycles	Presets • 251 • 151 • 101 • 51 • type a value between 1 and 251 for the SP flow cell type or between 1 and 151 for all other types

Field	Value
Read 2 Cycles	Presets • 251 • 151 • 101 • 51 • type a value between 0 and 251 for the SP flow cell type or between 0 and 151 for all other types
Use Custom Read 1 Primer	Select if applicable.
Use Custom Read 2 Primer	Select if applicable.
Use Custom Index Read 1 Primer	Select if applicable.
Reverse Complement Workflow	Select Yes (by default) for v1.5 reagents in NovaSeq Control Software v1.7 and onwards. Select No for v1 reagents.
UMI - Read 1 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 1. Leave blank if not applicable.
UMI - Read 2 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 2. Leave blank if not applicable.
UMI - Read 1 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.
UMI - Read 2 Start From Cycle	Enter the cycle number that Read 2 starts from. Leave blank if not applicable.
Sample Sheet Path	Automatically populated after the sample sheet has been generated.
Library Tube Barcode	Scan the library tube barcode.

4. Select Generate Sample Sheet and Run Recipe. This selection triggers the automation script, which:
   - Generates and attaches the sample sheet and JSON files to the placeholders in the Files area of the Record Details screen.
   - Populates the Sample Sheet Path field with the path to the sample sheet file.
5. Select Next Steps.
   This selection triggers the Prepare Files for NovaSeq automation, which copies the sample sheet and run recipe files to the location specified during installation. The NovaSeq instrument software uses these files to set up the run.

6. On the Assign Next Steps screen, make sure the Next Step for samples is prepopulated with Mark protocol as complete.
7. Select Finish Step.

At this point in the workflow, the user interaction ends. The flow cell is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) step.

Proceed to Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3).

Do not add samples to the Ice Bucket or start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) step. The integration does this action automatically.

Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3)

This protocol contains a single fully automated step - AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3).

The integration starts the step automatically and data from the run is parsed back into Clarity LIMS. No user interaction is required. However, you can open and review the various stages of the step in Clarity LIMS.

How the Integration Works

1. When initiating a run on the NovaSeq instrument, the NovaSeq Control Software (NVCS) looks for the sample sheet and run recipe (*.json file) on the shared network drive. The NVCS copies the sample sheet to the run directory.
   The NVCS uses the run recipe to start the sequencing run.
2. When the run starts, the instrument software creates a new run folder on the shared network drive and copies the following files into that folder:
   - `RunParameters.xml`
   - `RunInfo.xml`
   - LIMS/ <ContainerID>.json file (run recipe)
   - The Real-Time Analysis v3 (RTA3) software creates the InterOp folder.
3. The sequencing service detects the presence of the `RunParameters`, `RunInfo`, and JSON files and starts the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) step in Clarity LIMS.
4. As the run progresses, the InterOp data files are filled in.
5. At the end of the run, the NovaSeq creates the `CopyComplete.txt` file in the InterOp folder, which indicates to the sequencing service that the run has completed and the data files are ready.
6. The NovaSeq copies the run data files to the InterOp folder and the sequencing service triggers the Read InterOp Metrics automation. This automation reads the files, records the parsed metrics into Clarity LIMS, and completes the step in Clarity LIMS.

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Parse Run Parameters and Generate Link to Run Folder Automation

The Parse Run Parameters and Generate Link to Run Folder automation is automatically triggered on entry to the Record Details screen. This automation parses the `RunParameters.xml` file attached on the Step Setup screen and populates the global/master step fields with extracted values. For details on this automation, including how the parsed fields map to the master step fields in Clarity LIMS, refer to *NovaSeq 6000 Integration v2.5 Configuration & User Interaction* on page 915.

Read InterOp Metrics Automation

Automatically triggered when the sequencing service detects the `CopyComplete.txt` file has been created, this automation parses the InterOp data generated by the run.

The `read_interop_metrics` script records summary metrics into the following global fields in Clarity LIMS. The script fails if any field is not configured or cannot be written to.

- Yield PF (Gb) R1
- Yield PF (Gb) R2
- Reads PF (M) R1
- Reads PF (M) R2
- Cluster Density (K/mm^2) R1
- Cluster Density (K/mm^2) R2
- %PF R1
- %PF R2
- % Bases >=Q30 R1
- % Bases >=Q30 R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- % Aligned R1
- % Aligned R
- % Error Rate R1
- % Error Rate R2

For details, refer to *NovaSeq 6000 Integration v2.5 Configuration & User Interaction* on page 915.

Set Next Steps Automation

After the run metrics are parsed successfully, the Set Next Steps automation sets the next step for all samples to Mark protocol as complete. This step is completed automatically. For details, refer to *NovaSeq 6000 Integration v2.5 Configuration & User Interaction* on page 915.

Troubleshooting

If an automation trigger does not appear to run its corresponding scripts, see the following sections in the Clarity LIMS documentation:

- *Troubleshooting Automated Informatics*
- *Troubleshooting Automation in the API*

If an error occurs that does not provide direction on how to proceed, complete the following steps:

1. Confirm the version of the installed Illumina NovaSeq Integration Package by running the `rpm -qa | grep -i novaseq` command as follows.
   - For an on-premise deployment, run the command on Clarity LIMS server command line. This command retrieves the version of both RPMs installed.
   - For a cloud hosted deployment, run the command on the local server installation. This command retrieves the version of the remote extensions RPM installed.
2. If the error is related to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) step, there are two places to check for log file information:
   - If the step does not start, check the `NovaSeqIntegrator.log` file written to:

```
/opt/gls/clarity/extensions/novaseq/SequencingService/NovaSeqIntegrator.log
```

     This log file is written to this path wherever the remote extensions RPM was installed.
   - If the step starts but does not complete, open the step in Clarity LIMS (it can be found via a search). On the Record Details screen, the log file is attached to a placeholder called Log File. If you are unable to reach the Record Details screen, or if the file does not contain an error, review the `NovaSeqIntegrator.log` described previously.
3. Contact Illumina Support and supply the relevant information from the troubleshooting steps already performed.

NovaSeq 6000 Integration v2.5 Installation

The Illumina NovaSeq 6000 Integration v2.5 supports the integration between Clarity LIMS and the NovaSeq 6000 instrument. This integration also provides support for Clarity LIMS v6.2 and includes updates to the technology stack and third party libraries and utilities.

Clarity LIMS is automatically stopped during installation of the RPM and will need to be restarted.

The file-based and API-based integrations cannot run at the same time. When the API-based integration has finished installing, shut down Clarity LIMS and uninstall the file-based integration.

This document provides instructions for installing NovaSeq 6000 Integration v2.5. It also describes the components that are installed in the default configuration.

Integration Overview

Illumina NovaSeq 6000 Integration v2.5 includes the following features and functionality:

- Preconfigured NovaSeq 6000 v2.3 workflow that maps to lab protocols and instrument runs.
- The following preconfigured protocols:
  - NovaSeq Standard (NovaSeq 6000 v2.3) (supports standard library placement)
  - NovaSeq Xp (NovaSeq 6000 v2.3) (supports individual lane loading on the NovaSeq 6000)
- Automated generation of the sample sheet. This sample sheet is used with the bcl2fastq2 v2.20 analysis software.
- Automated generation of the run recipe file in the JSON format. This file is automatically uploaded to the instrument and is used to set up and initiate the run.
- Automated tracking of the following information for the NovaSeq 6000 sequencing run and parsing of run statistics into Clarity LIMS:
  - Progress and metrics of sequencing run
  - Per-instrument sequencing runs (tracked as part of the Run ID field value)
  - Sequencing run parameters
  - Real-Time Analysis v3 (RTA3) version and other run-specific information
- Preconfigured NovaSeq Validation Library Prep (NovaSeq 6000 v2.3) workflow used for validation purposes. This workflow contains a single-step protocol that models the library prep required to produce normalized libraries that are ready for the NovaSeq 6000 v2.3 workflow. For more information, refer to *NovaSeq 6000 Integration v2.5 Validation and Troubleshooting* on page 972.

Supporting Documentation

- For details on installed protocols and steps, automations, generated and captured files, and rules and constraints, refer to *NovaSeq 6000 Integration v2.5 Configuration & User Interaction* on page 915.
- For information on validating and troubleshooting the integration, refer to *NovaSeq 6000 Integration v2.5 Validation and Troubleshooting* on page 972.

Prerequisites

NovaSeq 6000 Integration v2.5 has the following prerequisites:

- Mount run data network-attached storage (NAS) share
- Secret Util is installed
- IPP is installed

Prerequisite 1: Mount Run Data NAS Share

Mounting the NAS share of run data is needed to capture and generate files associated with the sequencing run. To mount NAS shares that contain data from the Clarity LIMS server, set up the following Write privileges as the glsai user:

- Sample sheet directory—This directory is where the Prepare Files for NovaSeq script writes sample sheets.
- Run recipe directory—This directory is where the Prepare Files for NovaSeq script writes run recipe files in the JSON format.

Set up the Read privileges as the glsjboss and glsai users (or as the entire claritylims group) for the run output directory. This directory is where the sequencing service looks for run folders. The run folders must be in this directory. The service does not search sub-directories.

The sample sheet, run recipe, and run output directories can be configured independently and do not have to be on the same mount or share.

Prerequisite 2: Secret Util Installation

The Clarity LIMS installation tooling configures the Secret Util installation. No additional configuration is necessary. For more information on Secret Utility configuration, refer to the *Clarity LIMS Core Functionality* documentation.

Installation

NovaSeq 6000 Integration v2.5 supports both on-premise and cloud integrations. This integration is distributed as the following RPM packages:

- BaseSpaceLIMS-novaseq-extensions
- BaseSpaceLIMS-novaseq-sequencing-service (depends on BaseSpaceLIMS Automation Worker v1.x)

The BaseSpaceLIMS-novaseq-extensions RPM installs the following items:

- Protocols and steps
- Validation workflow
- Database properties that configure the service

- `novaseq-extensions.jar` file that provides sample sheet generation and other utility scripts

The BaseSpaceLIMS-novaseq-sequencing-service RPM installs the following items:

- Bash scripts to run the novaseq_seqservice through systemctl
- `novaseq-sequencing.jar`
- `novaseq-remote-extensions.jar` file that contains scripts that need access to the run folders on the NAS

On-Premise Installation

Use the following instructions to install the BaseSpaceLIMS-novaseq-extensions and BaseSpaceLIMS-novaseq-sequencing-service RPMs on the Clarity LIMS server.

Install the RPMs

1. On the Clarity LIMS server, log in as the root user.
2. Run the following yum commands to install the RPMs:

   > You must include the --enablerepo command line argument. Illumina Support provides the repo file and appropriate name to use.

```
yum install BaseSpaceLIMS-novaseq-extensions --enablerepo=<< repo name info from support >>
yum install BaseSpaceLIMS-novaseq-sequencing-service --enablerepo=<< repo name info from support >>
```

3. Enter `y` to confirm that you want to proceed with the RPM installations.
4. If Secret Util is not installed or configured, you must configure it before proceeding. As the glsjboss user, run the following script:

```
bash /opt/gls/clarity/config/pending/05_configure_claritylims_secretutil.sh
```

   For more information on installing Secret Util with the Integration Module, refer to *Install/Upgrade Secret Management for Integration Modules* in the *Clarity LIMS Core Functionality* documentation.

   > For the on-premise installation, the Illumina Vault server is not available for public access.
   >
   > In the configuration, make sure the system passwords are configured correctly in File mode.

5. [Optional] If you are upgrading from a previous integration, check for any usages of the Custom API Username in the current system as follows.
   a. Install ClarityLIMS-UpgradePreValidation.
   b. Check for any custom API username requirements (eg, novaseq_user).
   Typically, the default API user name is apiuser.
   c. Run the following script:

```
/opt/gls/ClarityUpgradeValidation/bin/checkUsernameConfiguration.sh
```

   d. After the script is complete, check that a custom user is configured in the results.

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If a custom API username is configured, a warning message displays.

e. If a custom API username is required, configure the username and password using Secret Util:

```
java -jar /opt/gls/clarity/tools/secretutil/secretutil.jar -n=INTEGRATION -
u=<password> <key>
```

For a custom API username, set the `<key>` to `apiusers/{custom API username}` (eg, `apiusers/novaseq_user`). For more information on Secret Utility configuration, refer to the *Clarity LIMS Core Functionality* documentation.

f. Use the following command to make sure that the password is saved correctly in Secret Util:

```
java -jar /opt/gls/clarity/tools/secretutil/secretutil.jar -n=INTEGRATION
<key>
```

Import Workflow Configurations for NovaSeq 6000

1. As the glsjboss user, use the following command to run the `configure_extensions_novaseq.sh` script:

```
bash /opt/gls/clarity/config/configure_extensions_novaseq.sh
```

2. When prompted, enter the channel name for the Automation Worker and sequencing service.

When upgrading from NovaSeq Integration v2.2 to v2.5, a conflict is expected during the workflow import with the Flowcell Type analyte custom field due to SP being added to the list of preset values. You can manually add `<preset>SP</preset>` as one of the values of the Flowcell Type custom field after you import the workflow.

Configure the Database Service Properties

To enable capture and generation of files associated with the sequencing run, configure the following properties using the omxProps-ConfigTool utility.

Table 237 Configurable Database Properties

Property	Description
novaseq.runSetupFolder	The directory where the Prepare Files for NovaSeq script writes run recipe files in the JSON format. This must be an existing folder and must be writable by the glsai user. For more information, refer to *Prerequisites* on page 990.

Property	Description
novaseq.sampleSheetPathPrefixSearch.SUFFIX	The directory where NVCS or analysis software looks for sample sheets. This directory is typically a Windows path or a network drive. The integration does not use this path directly, but the attachment filed in the run recipe file is determined by adding the sample sheet file name to this value.
novaseq.sampleSheetPathPrefixReplace.SUFFIX	The directory where the Prepare Files for NovaSeq script writes sample sheets. This must be an existing folder and must be writable by the glsai user. For more information, refer to *Prerequisites* on page 990.
novaseq.sampleSheetPathPrefixSearchReplaceSuffixes	This property must be set to the SUFFIX used in the novaseq.sampleSheetPathPrefixSearch.SUFFIX and novaseq.sampleSheetPathPrefixReplace.SUFFIX properties.
novaseq.seqservice.netPathPrefixSearch.SUFFIX	This property is the search prefix used to map the OutputRunFolder field in the `RunParameters.xml` file to the corresponding Linux directory. The OutputRunFolder is converted by replacing this string with the netPathPrefixReplace value. The value of this property is also in the output_folder field of the run recipe file.

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Property	Description
novaseq.seqservice.netPathPrefixReplace.SUFFIX	The directory where the sequencing service looks for run folders. This must be an existing folder and must be writable by the glsai or glsjboss users, or by the claritylims group. For more information, refer to *Prerequisites* on page 990. The run folders must be in the configured directory. The service does not search sub-directories.
novaseq.seqservice.netPathPrefixSearchReplaceSuffixes	This property must be set to the single SUFFIX used in novaseq.seqservice.netPathPrefixSearch.SUFFIX and novaseq.seqservice.netPathPrefixReplace.SUFFIX.

This version of the integration only supports a single active search-replace pair of each type. The sampleSheetPathPrefixSearchReplaceSuffixes and netPathPrefixSearchReplaceSuffixes properties must contain a single number instead of a comma-separated list of numbers.

The sample sheet, run recipe, and run output folders can all be configured independently and do not have to be on the same mount or share. For examples of valid configuration mount points and properties, refer to *Example Network Folder Structure and Database Properties* on page 1000.

Start the Sequencing Service

1. Run the following command to start the sequencing service:

```
systemctl start novaseq_seqservice
```

Cloud Installation

The BaseSpaceLIMS-novaseq-extensions RPM must be installed on the Clarity LIMS server. The BaseSpaceLIMS-novaseq-sequencing-service RPM can be installed remotely on another server within the network.

Specifications

The following hardware, operating system, and network specifications must be met to install the BaseSpaceLIMS-novaseq-sequencing-service RPM:

- Hardware requirements:
  - 64-bit processor (dual core 2.0 GHz)
  - OS requirements, plus at least an additional 2 GB RAM
  - A minimum of 5 GB of hard disk space

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- Operating system requirements:
  - Clarity LIMS v6.2 or later
  - Oracle Linux (for compatibility, refer to *NovaSeq 6000 Integration v2.5 Release Notes* on page 1002)
  - Minimum requirements for remote Automation Worker (for more information, refer to the *Clarity LIMS Core Functionality* documentation)

  > If you are running the sequencing service and Automation Worker on the same instance, make sure that the instance is running a compatible version of Oracle Linux. For compatibility, refer to *NovaSeq 6000 Integration v2.5 Release Notes* on page 1002.

- Network requirements:
  - SSL access to the Clarity LIMS server from the network
  - The mounted folder where the run recipes are written
  - The mounted folder where the sample sheets are written
  - The mounted folder where the sequencing runs are written

Install the RPM

1. On the applicable server, log in as the root user.
2. Run the following yum command to install the BaseSpaceLIMS-novaseq-extensions RPM:

   > You must include the --enablerepo command line argument. Illumina Support provides the repo file and appropriate name to use.

```
yum install BaseSpaceLIMS-novaseq-extensions --enablerepo=<< repo name info from support >>
```

3. Enter `y` to confirm that you want to proceed with the RPM installation.
4. If Secret Util is not installed or configured, you must configure it before proceeding. As the glsjboss user, run the following script:

```
bash /opt/gls/clarity/config/pending/05_configure_claritylims_secretutil.sh
```

   For more information on installing Secret Util with the Integration Module, refer to *Install/Upgrade Secret Management for Integration Modules* in the *Clarity LIMS Core Functionality* documentation.

5. [Optional] If a custom API username is required, configure the username and password as follows.
   a. Run the following command using Secret Util:

```
java -jar /opt/gls/clarity/tools/secretutil/secretutil.jar -n=INTEGRATION -u=<password> <key>
```

      For a custom API username, set the `<key>` to `apiusers/{custom API username}` (eg, `apiusers/novaseq_user`). For more information on Secret Utility configuration, refer to the *Clarity LIMS Core Functionality* documentation.

   b. Use the following command to make sure that the password is saved correctly in Secret Util:

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```
java -jar /opt/gls/clarity/tools/secretutil/secretutil.jar -n=INTEGRATION
<key>
```

Import Workflow Configurations for NovaSeq 6000

1. As the glsjboss user, use the following command to run the `configure_extensions_novaseq.sh` script:

```
bash /opt/gls/clarity/config/configure_extensions_novaseq.sh
```

2. When prompted, enter the channel name for the Automation Worker and sequencing service. The recommended channel name is remotens. For more information, refer to *Update Channel Name on page 996*.

When upgrading from NovaSeq Integration v2.2 to v2.5, a conflict is expected during the workflow import with the Flowcell Type analyte custom field due to SP being added to the list of preset values. You can manually add `<preset>SP</preset>` as one of the values of the Flowcell Type custom field after you import the workflow.

Update Channel Name

The configure_extensions_novaseq.sh script prompts for a channel name and sets the name on the following preconfigured automations:

- Prepare Files for NovaSeq (configured on Dilute and Denature (NovaSeq 6000 v2.3) and Load to Flowcell (NovaSeq 6000 v2.3) steps)
- Read InterOp Metrics (configured on AUTOMATED NovaSeq Run (NovaSeq 6000 v2.3) step)

If you need to change the channel name, you must update it manually on each automation. The import script does not update this setting.

Configure the Database Service Properties

To enable capture and generation of files associated with the sequencing run, configure the following properties using the omxProps-ConfigTool utility.

Table 238 Configurable Database Properties

Property	Description
novaseq.runSetupFolder	The directory where the Prepare Files for NovaSeq script writes run recipe files in the JSON format. This must be an existing folder and must be writable by the glsai user. For more information, refer to *Prerequisites on page 990*.

For Research Use Only. Not for use in diagnostic procedures.

Property	Description
novaseq.sampleSheetPathPrefixSearch.SUFFIX	The directory where NVCS or analysis software looks for sample sheets. This directory is typically a Windows path or a network drive. The integration does not use this path directly, but the attachment filed in the run recipe file is determined by adding the sample sheet file name to this value.
novaseq.sampleSheetPathPrefixReplace.SUFFIX	The directory where the Prepare Files for NovaSeq script writes sample sheets. This must be an existing folder and must be writable by the glsai user. For more information, refer to *Prerequisites* on page 990.
novaseq.sampleSheetPathPrefixSearchReplaceSuffixes	This property must be set to the SUFFIX used in the novaseq.sampleSheetPathPrefixSearch.SUFFIX and novaseq.sampleSheetPathPrefixReplace.SUFFIX properties.
novaseq.seqservice.netPathPrefixSearch.SUFFIX	This property is the search prefix used to map the OutputRunFolder field in the RunParameters.xml file to the corresponding Linux directory. The OutputRunFolder is converted by replacing this string with the netPathPrefixReplace value. The value of this property is also in the output_folder field of the run recipe file.

Property	Description
novaseq.seqservice.netPathPrefixReplace.SUFFIX	The directory where the sequencing service looks for run folders. This must be an existing folder and must be writable by the glsai or glsjboss users, or by the claritylims group. For more information, refer to *Prerequisites* on page 990. The run folders must be in the configured directory. The service does not search sub-directories.
novaseq.seqservice.netPathPrefixSearchReplaceSuffixes	This property must be set to the single SUFFIX used in novaseq.seqservice.netPathPrefixSearch.SUFFIX and novaseq.seqservice.netPathPrefixReplace.SUFFIX.

This version of the integration only supports a single active search-replace pair of each type. The sampleSheetPathPrefixSearchReplaceSuffixes and netPathPrefixSearchReplaceSuffixes properties must contain a single number instead of a comma-separated list of numbers.

The sample sheet, run recipe, and run output folders can all be configured independently and do not have to be on the same mount or share. For examples of valid configuration mount points and properties, refer to *Example Network Folder Structure and Database Properties* on page 1000.

Install Sequencing Service RPM on Remote Server

1. On the applicable server, log in as the root user.
2. Run the following yum command to install the RPM:

```
yum install BaseSpaceLIMS-novaseq-sequencing-service
```

3. Enter `y` to confirm that you want to proceed with the RPM installation.

Set Up API Connection Properties File

1. Run the following script to set up the API connection properties file at `/opt/gls/clarity/automation_worker/conf/api-access.properties`:

```
bash /opt/gls/clarity/automation_worker/node/bin/configure.sh
```

   The `configure.sh` script is provided by BaseSpaceLIMS-AutomationWorker.

Start the Sequencing Service

1. Run the following command to start the sequencing service:

```
systemctl start novaseq_seqservice
```

Workflows, Protocols, and Steps Installed

NovaSeq 6000 Integration v2.5 includes the workflows, protocols, and steps listed in the following table.

Workflow	Protocols	Steps
NovaSeq 6000 v2.3	Run Format (NovaSeq 6000 v2.3)	1. Define Run Format (NovaSeq 6000 v2.3)
	NovaSeq Standard (NovaSeq 6000 v2.3)	1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.3) 2. Dilute and Denature (NovaSeq 6000 v2.3)
	NovaSeq Xp (NovaSeq 6000 v2.3)	1. Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.3) 2. Dilute, Denature & ExAmp (NovaSeq 6000 v2.3) 3. Load to Flowcell (NovaSeq 6000 v2.3)
	AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3)	1. AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3)
NovaSeq Validation Library Prep (NovaSeq 6000 v2.3)	NovaSeq Validation Library Prep (NovaSeq 6000 v2.3)	1. NovaSeq Validation Library Prep (NovaSeq 6000 v2.3)

For detailed descriptions of the steps and automations included in each protocol, and details on other components in the configuration, refer to *NovaSeq 6000 Integration v2.5 Configuration & User Interaction* on page 915.

For instructions on using the NovaSeq Validation Library Prep (NovaSeq 6000 v2.3) protocol, refer to *NovaSeq 6000 Integration v2.5 Validation and Troubleshooting* on page 972.

The configuration provided with this integration has been established to support NovaSeq 6000 lab processes. Any configuration changes to protocols or workflows, including renaming protocols, steps, and fields, could break processes.

Instrument Software

The NovaSeq instrument software includes the following components:

- NovaSeq Control Software (NVCS)—Contains the user interface for setting up the sequencing run. Responsible for controlling the instrument and acquiring the images.

- Real-Time Analysis v3 (RTA3)—Takes the images generated by the first module, processes, and analyzes them. Makes sure that data files are created and copied to the final destination folder.
- Sequencing Analysis Viewer (SAV)—Displays the important quality metrics generated by the RTA3 software.
- NovaSeq recipes—Provides system operation instructions for use with NovaSeq 6000 reagent kits for SP, S1, S2, and S4 flow cells.
- Universal Copy Service—Copies output files to destinations such as final destination folder and/or BaseSpace Sequence Hub (when the instrument is configured for use with BaseSpace Sequence Hub).

Instrument Integration Configuration

For the NovaSeq API integration to work, the NovaSeq instrument must be able to communicate with Clarity LIMS through the API. Complete the following steps to configure the NVCS and confirm that you can access Clarity LIMS from the instrument.

1. Launch NVCS and wait for the initialization process to complete.
2. On the NVCS Settings page, do the following:
   a. Under Mode Selection, select File-Based.
   b. In the adjacent field, enter the network folder location that NVCS retrieves the JSON recipe from.
   c. In the Output Folder field, enter the default output folder location.
3. Select Save to complete the configuration.
4. On the NVCS home page, you can now select Sequence to log into Clarity LIMS and start a run.

Example Network Folder Structure and Database Properties

Instrument Control Software Configuration

Refer to the following examples for the configuration of the instrument control software:

- Sample sheet directory: `\\nas\novaseq\sample_sheets`
- JSON run recipes directory: `\\nas\novaseq\run_setup`
- Output directory: `\\nas\novaseq\runs`

Linux Server Mount Points

Refer to the following examples for the Linux server mount points on either the Clarity LIMS or sequencing service server:

- Sample sheet directory: `/mnt/novaseq_sample_sheets`
- JSON run recipes directory: `/mnt/novaseq_run_setup`
- Output directory: `/mnt/novaseq_runs`

Clarity LIMS Configuration Properties

Refer to the following examples for the Clarity LIMS configuration properties:

- `novaseq.runSetupFolder`: `/mnt/novaseq_run_setup`
- `novaseq.sampleSheetPathPrefixSearchReplaceSuffixes`: 1
- `novaseq.sampleSheetPathPrefixSearch.1`: `\\nas\novaseq\sample_sheets`
- `novaseq.sampleSheetPathPrefixReplace.1`: `/mnt/novaseq_sample_sheets`
- `novaseq.seqservice.netPathPrefixSearchReplaceSuffixes`: 1
- `novaseq.seqservice.netPathPrefixSearch.1`: `\\nas\novaseq\runs`
- `novaseq.seqservice.netPathPrefixReplace.1`: `/mnt/novaseq_runs`

Network Folder Structure and Contents

The following examples assume the same configuration and mount points from the previous sections.

After completing the Dilute and Denature (NovaSeq 6000 v2.3) or Load to Flowcell (NovaSeq 6000 v2.3) step, an automated script copies the run recipe JSON file and sample sheet to the appropriate network locations. For example, a library tube or flow cell with the barcode AA12345-NNN would have the following directory content:

- `/mnt/novaseq_run_setup/AA12345-NNN.json` (run recipe file)
- `/mnt/novaseq_sample_sheets/AA12345-NNN.csv` (sample sheet)

When the NovaSeq run starts, the instrument control software creates a new run folder named after the run ID. Then, the software copies the run recipe JSON file to a Clarity LIMS subfolder inside it. For example, a run ID of 170301_A001_AHHCC would have the following directory content:

- `/mnt/novaseq_run_setup/AA12345-NNN.json`
- `/mnt/novaseq_sample_sheets/AA12345-NNN.csv`
- `/mnt/novaseq_runs/170301_A001_AHHCC/LIMS/AA12345-NNN.json`
- `/mnt/novaseq_runs/170301_A001_AHHCC/RunInfo.xml`
- `/mnt/novaseq_runs/170301_A001_AHHCC/RunParameters.xml`

The NovaSeq 6000 sequencing service detects the run folder based on the presence of the three files inside it and starts the AUTOMATED NovaSeq Run (NovaSeq 6000 v2.3) step automatically.

After the sequencing run has completed, the Universal Copy Service copies the run data and InterOp files to the network drive. Then, the service creates a `CopyComplete.txt` file in the run folder. At this point, the directory would have the following content:

- `/mnt/novaseq_run_setup/AA12345-NNN.json`
- `/mnt/novaseq_sample_sheets/AA12345-NNN.csv`
- `/mnt/novaseq_runs/170301_A001_AHHCC/LIMS/AA12345-NNN.json`
- `/mnt/novaseq_runs/170301_A001_AHHCC/RunInfo.xml`

- `/mnt/novaseq_runs/170301_A001_AHHCC/RunParameters.xml`
- `/mnt/novaseq_runs/170301_A001_AHHCC/InterOp`
- `/mnt/novaseq_runs/170301_A001_AHHCC/CopyComplete.txt`

The NovaSeq 6000 sequencing service detects the completed sequencing run based on the presence of the `CopyComplete.txt` file, executes InterOp metrics parsing for the run, and automatically completes the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) step.

NovaSeq 6000 Integration v2.5 Release Notes

These Release Notes describe the key changes to software components for NovaSeq 6000 Integration Package v2.5.

Compatibility

This version of the integration package is compatible with the following software:

- Clarity LIMS v6.2 and later
- NGS Extensions Package v5.23 and later
- NovaSeq Control Software (NVCS) v1.8.0
- Illumina bcl2fastq v2.20
- Oracle Linux (64-bit) v8.8

New Features

- Updates Java and third-party dependency libraries.
- Updates Groovy to v3.0.7.

Defects Fixed

None

Known Issues

Log file name is missing the `-Logfile.html` suffix and the content is missing the sample sheet generation logs.

NovaSeq 6000 Integration v2.4.1 Configuration & User Interaction

The Illumina NovaSeq 6000 Integration Package v2.4.1 supports the integration of Clarity LIMS to Illumina NovaSeq 6000 instruments.

This document describes the integration and includes information about the preconfigured protocols, steps, automations, installed components, configuration requirements, rules, and constraints.

For instructions on validating and troubleshooting the Illumina NovaSeq 6000 Integration, refer to *NovaSeq 6000 Integration v2.4.1 Validation and Troubleshooting* on page 1059.

The configuration provided in this integration has been established to support NovaSeq 6000 lab processes. Any configuration changes to protocols or workflows—including renaming protocols, steps, and fields—could break the process.

Integration Functionality

The integration includes the following functionality:

- Preconfigured NovaSeq 6000 v2.3 workflow that maps to lab protocols and instrument runs.
- Preconfigured NovaSeq Standard (NovaSeq 6000 v2.3) protocol that supports the loading of pooled libraries into a library tube.
- Preconfigured NovaSeq Xp (NovaSeq 6000 v2.3) protocol that supports individual lane loading on the NovaSeq.
- Automated generation of a sample sheet for use with bcl2fastq2 v2.20 analysis software.
- Automatic generation of run recipe file (JSON format) that is automatically uploaded to the sequencing instrument and used to set up and initiate the run.
- Automated tracking of the NovaSeq sequencing run and parsing of run statistics (per run per lane) into Clarity LIMS, including:
  - Progress and metrics of sequencing run
  - Per instrument sequencing runs (tracked as part of the Run ID field value)
  - Sequencing run parameters
  - Real-Time Analysis v3 (RTA3) run directory location and other run-specific information
- Preconfigured NovaSeq Validation Library Prep (NovaSeq 6000 v2.3) workflow used for validation purposes only. The workflow contains a single-step protocol that models the library prep required to produce normalized libraries that are ready for the NovaSeq 6000 v2.3 workflow. For details, refer to *NovaSeq 6000 Integration v2.4.1 Validation and Troubleshooting* on page 1059.

Prerequisites and Assumptions

It is assumed that samples enter the NovaSeq 6000 v2.3 workflow as normalized libraries. That is, before they are assigned to the workflow the following actions occur:

- Samples have been accessioned into Clarity LIMS.
- Samples have been run through QC and library prep.
- Samples have been normalized, and the value is captured in a field called Normalized Molarity (nM).

For more information on sample accessioning, refer to *Sample Accessioning* and *Upload and Modify Samples* in the Getting Started section of the Clarity LIMS Core Functionality documentation.

You can assign samples to workflows automatically, using a routing script, or manually—from the Projects & Samples dashboard. Refer to *Assign and Process Samples* in the Clarity LIMS Core Functionality documentation.

Workflows, Protocols, and Steps

The Illumina NovaSeq 6000 Integration Package v2.4.1 includes two workflows:

- NovaSeq 6000 v2.3
- NovaSeq Validation Library Prep (NovaSeq 6000 v2.3)

The following table lists and describes the protocols and steps included in these workflows.

Table 239 NovaSeq 6000 Integration Package v2.4.1 Protocols and Steps

Protocol Name	Protocol Purpose	Protocol Steps
NovaSeq Validation Library Prep (NovaSeq 6000 v2.3) workflow		
NovaSeq Validation Library Prep (NovaSeq 6000 v2.3)	Included for validation purposes only, this protocol models the library prep steps required to advance samples to the Run Format (NovaSeq 6000 v2.3) protocol. The protocol contains a single step: NovaSeq Validation Library Prep (NovaSeq 6000 v2.3). At the end of this step, a routing script sends the samples to the first step of the NovaSeq 6000 v2.3 workflow, which is Define Run Format (NovaSeq 6000 v2.3).	NovaSeq Validation Library Prep (NovaSeq 6000 v2.3)
NovaSeq 6000 v2.3 workflow		
Run Format (NovaSeq 6000 v2.3)	Allows for the assignment of per sample values for Loading Workflow Type, Normalized Molarity, Flowcell Type, and Final Loading Concentration (pM). • Loading Workflow Type: Select from NovaSeq Standard or NovaSeq Xp. • Normalized Molarity: Enter a value for each sample. • Flowcell Type: Select from options S1, S2, S4, and SP. • Final Loading Concentration (pM): Select from the options 225 (PCR-free workflows) or 400 (Nano workflows). Alternatively, enter a different value. Compares the Normalized Molarity value of each sample with the Minimum Molarity value. Routing script sends samples to the NovaSeq Standard or NovaSeq Xp protocol, according to the selected Loading Workflow Type. Samples with Normalized Molarity less than Minimum Molarity are removed from the workflow.	Define Run Format (NovaSeq 6000 v2.3)

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Protocol Name	Protocol Purpose	Protocol Steps
NovaSeq Standard (NovaSeq 6000 v2.3)	Samples are pooled and added to the library tube in preparation for the NovaSeq run. Sample sheet and run recipe files are generated. Routing script sends library tube to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) protocol.	Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.3) Dilute and Denature (NovaSeq 6000 v2.3)
NovaSeq Xp (NovaSeq 6000 v2.3)	Samples are pooled and added to lanes on the NovaSeq flow cell. The flow cell type is determined by the option selected in the Define Run Format (NovaSeq 6000 v2.3) step. Sample sheet and run recipe files are generated. Samples are queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) protocol.	Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.3) Dilute, Denature, and ExAmp (NovaSeq 6000 v2.3) Load to Flowcell (NovaSeq 6000 v2.3)
AUTOMATED - NovaSeq Run (NovaSeq6000 v2.3)	All samples complete the workflow by going through this protocol. This protocol contains one fully automated step. Do not add samples to the Ice Bucket or start the step. The integration does this action automatically.	AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3)

Validation Workflow

The NovaSeq Validation Library Prep (NovaSeq 6000 v2.3) workflow allows for validation of the system after installation is complete. For details, refer to *NovaSeq 6000 Integration v2.4.1 Validation and Troubleshooting* on page 1059.

Step Automations

The following table lists the step automations included in the NovaSeq 6000 v2.3 workflow. Automation details are provided in the sections following the table.

For details on the validation workflow, refer to *NovaSeq 6000 Integration v2.4.1 Validation and Troubleshooting* on page 1059.

Table 240 NovaSeq 6000 v2.3 Workflow Step Automations

Protocol	Step	Automation
1. Run Format (NovaSeq 6000 v2.3)	1. Define Run Format (NovaSeq 6000 v2.3)	• Routing Script • Set Next Step
2. NovaSeq Standard (NovaSeq 6000 v2.3)	1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.3)	• Calculate Volumes • Set Next Step • Validate Inputs Flowcell Type and Single Pool • Validate Unique Indexes Not used. This functionality is handled by the core Clarity LIMS configuration for pooling.
	2. Dilute and Denature (NovaSeq 6000 v2.3)	• Generate Sample Sheet and Run Recipe • Prepare Files for NovaSeq • Routing Script • Validate Library Tube Barcode • Validate Single Input
3. NovaSeq Xp (NovaSeq 6000 v2.3)	1. Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.3)	• Calculate Volumes • Set Next Step • Validate Inputs Flowcell Type and Single Pool • Validate Unique Indexes Not used. This functionality is handled by the core Clarity LIMS configuration for pooling.
	2. Dilute, Denature, and ExAmp (NovaSeq 6000 v2.3)	• Calculate Volumes • Set Next Step • Validate Inputs Flowcell Type
	3. Load to Flowcell (NovaSeq 6000 v2.3)	• Generate Sample Sheet and Run Recipe • Prepare Files for NovaSeq • Validate Flowcell Barcode • Validate Inputs and Selected Container

Protocol	Step	Automation
4. AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3)	1. AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3)	• Parse Run Parameters and Generate Link to Run Folder • Read InterOp Metrics • Set Next Steps • Update Lane number

Terminology

The Illumina NovaSeq 6000 Integration Package v2.4.1 supports Clarity LIMS v5 and later.

As of Clarity LIMS v5, the term user-defined field (UDF) has been replaced with custom field in the user interface. However, the API resource is still called UDF.

There are two types of custom fields:

- Master step fields—Configured on master steps. Master step fields only apply to the following:
  - The master step on which the fields are configured.
  - The steps derived from those master steps.
- Global fields—Configured on entities (eg, submitted sample, derived sample, measurement, etc.). Global fields apply to the entire Clarity LIMS system.

Protocol 1: Run Format (NovaSeq 6000 v2.3)

This protocol sets the Loading Workflow Type and allows you to choose the appropriate Flowcell Type and Final Loading Concentration(pM). At the end of the protocol, a routing script sends the normalized libraries to either the NovaSeq Standard (NovaSeq 6000 v2.3) or the NovaSeq Xp (NovaSeq 6000 v2.3) protocol.

This protocol contains one step: Define Run Format (NovaSeq 6000 v2.3).

Step 1: Define Run Format (NovaSeq 6000 v2.3)

Step input: NTP (normalized libraries)

Step output: None

Set Next Steps Automation

Automatically triggered on exit of the Record Details screen, this automation does the following actions:

- Sets the next step for samples to REMOVE with the `nextStep = ::REMOVE::` formula.
- Calculates the Minimum Molarity using the following formula:

```
input.::Minimum Molarity (nM):: = (5 * input.::Final Loading Concentration
(pM)::)/1000
```

- Checks Normalized Molarity value. For samples with no Normalized Molarity value (eg, an empty value, not including 0), the automation generates an error message stating that the field cannot be empty:

```
if (!input.hasValue(::Normalized Molarity (nM)::)) { fail(::The Normalized Molarity cannot be empty.::) ; }
```

- Compares the Normalized Molarity value of each sample with the Minimum Molarity value.
- If Normalized Molarity value is lower than the Minimum Molarity value, sets the Loading Workflow Type of the sample to [Remove from workflow] and records a message in the Warning field:

```
else if (input.::Normalized Molarity (nM):: < input.::Minimum Molarity (nM)::) { input.::Warning:: = ::The Normalized Molarity is too low.:: ; input.::Loading Workflow Type:: = ::[Remove from workflow]:: ; } else { input.::Warning:: = ::n/a:: }
```

At this point, there are two options:

- Correct the Normalized Molarity value on the Record Details screen. Also, edit the Loading Workflow Type field and set it to NovaSeq Standard or NovaSeq Xp, as applicable.
- Complete the protocol without correcting the Normalized Molarity value. In this case, those samples are removed from the workflow.

Routing Script Automation

Automatically triggered on exit of the step, this automation invokes the changeWorkflow script, which routes step inputs appropriately.

- Samples with Loading Workflow Type field value of NovaSeq Standard are routed to the NovaSeq 6000 v2.3 workflow and queued for the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.3) step.
- Samples with Loading Workflow Type field value of NovaSeq Xp are routed to the NovaSeq 6000 v2.3 workflow and queued for the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.3) step.

The default automation command line is as follows.

```
bash -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar
-u {username} -p {password} -i {stepURI:v2} -l {compoundOutputFileLuid0} script:changeWorkflow \
\
--FIELD_NAME 'Loading Workflow Type' \
--FIELD_VALUE 'NovaSeq Standard' \
--WORKFLOW 'NovaSeq 6000 v2.3' \
--STEP 'Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.3)' \
--INPUTS_OR_OUTPUTS 'INPUTS' \
```


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```
\
--FIELD_NAME 'Loading Workflow Type' \
--FIELD_VALUE 'NovaSeq Xp' \
--WORKFLOW 'NovaSeq 6000 v2.3' \
--STEP 'Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.3)' \
--INPUTS_OR_OUTPUTS 'INPUTS'"
```

Master Step Fields

The following table provides field configuration details for the fields defined on the Define Run Format (NovaSeq 6000 v2.3) step.

Table 241 Define Run Format (NovaSeq 6000 v2.3) Master Step Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Comment	Multiline Text		None
Instruction	Text	Read Only	Default • Add Flowcell Type and Loading Workflow Type below
Flowcell Type	Text Dropdown	Required Field Custom Entries	Hidden (does not show on Record Details screen) Presets • S1 • S2 • S3 • S4
Loading Workflow Type	Text Dropdown	Required Field	Hidden (does not show on Record Details screen) Presets • NovaSeq Standard • NovaSeq Xp

Global Fields

The following table lists the global fields that are configured to display on the Define Run Format (NovaSeq 6000 v2.3) step.

Table 242 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
Adjusted Per Sample Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Final Loading Concentration (pM)	Numeric Dropdown	Required Field Custom Entries	Presets • 225 • 400 Decimal places displayed = 0
Flowcell Type	Text Dropdown	Required Field	Presets • S1 • S2 • S4 • SP
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
Minimum Molarity (nM)	Numeric		Decimal places displayed = 2
Normalized Molarity (nM)	Numeric		Decimal places displayed = 2
Per Sample Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Warning	Text Dropdown	Read Only Custom Entries	Presets • The Normalized Molarity (nM) is too low. • Not applicable

User Interaction

1. On the Record Details screen in the Sample Details table, complete the following fields (values can vary across samples):
   - **Loading Workflow Type**—Select NovaSeq Standard or NovaSeq Xp from the drop-down list.
   - **Flowcell Type**—Select S1, S2, S4, or SP from the drop-down list.
   - **Final Loading Concentration (pM)**—Select from the two preset options (225 (for PCR-free workflows) or 400 (for Nano workflows)) or enter a different value.
   - **Normalized Molarity**—Enter a value for each sample.
2. Select Next Steps.
   This selection triggers the Next Steps automation, which does the following actions:
   - Sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step successfully.
   - Calculates the Minimum Molarity.
   - Checks Normalized Molarity value. For samples with no Normalized Molarity value (ie, an empty value, not including 0), the automation generates an error message stating that the field cannot be empty.
   - Compares the Normalized Molarity value of each sample with the Minimum Molarity value.
3. On the Assign Next Steps screen, observe the following changes:
   - In the Sample Details table, the Next Step for all samples is prepopulated with Remove from workflow—regardless of the Loading Workflow Type.

     > ⚠ Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.

   - For samples whose Normalized Molarity value is lower than the Minimum Molarity value, the Loading Workflow Type is set to Remove from workflow and a message is recorded in the Warning field for the sample.
4. Complete one of the following options:
   - Return to the Record Details screen and adjust the Normalized Molarity value so that it equals or exceeds the Minimum Molarity value. Also, set the Loading Workflow Type to NovaSeq Standard.
   - Complete the protocol without correcting the Normalized Molarity value. In this case, the samples in question are removed from the Clarity LIMS workflow.
5. Select Finish Step.
   The Routing Script automation is triggered when:

- Samples where Loading Workflow Type is set to Remove from workflow (eg, where Normalized Molarity value is lower than the Minimum Molarity) are removed from the Clarity LIMS workflow.
- Samples where Loading Workflow Type is set to NovaSeq Standard are routed to the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.3) step. This step is the first of two steps in the NovaSeq Standard (NovaSeq 6000 v2.3) protocol.
- Samples where Loading Workflow Type is set to NovaSeq Xp are routed to the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.3) step. This step is the first of three steps in the NovaSeq Xp (NovaSeq 6000 v2.3) protocol.

Protocol 2: NovaSeq Standard (NovaSeq 6000 v2.3)

Samples are routed to this protocol if the Loading Workflow Type value is set to NovaSeq Standard. Samples are pooled and added to a library tube in preparation for the NovaSeq run.

At the end of this protocol, a routing script sends the library tube to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) protocol.

This protocol contains two steps:

1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.3)
2. Dilute and Denature (NovaSeq 6000 v2.3)

Step 1: Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.3)

In this step, manually place libraries into a single pool. Resuspension buffer and reagents are added.

> Only create one pool per step.

Step input: NTP (normalized libraries)

Step output: Bulk pool

The following automations are configured on the step:

- Calculate Volumes
- Set Next Step
- Validate Inputs Flowcell Type and Single Pool
- Validate Unique Indexes (Not used. This functionality is handled by the core Clarity LIMS configuration for pooling)

These automations are described in the order in which they are triggered at run time.

Validate Inputs Flowcell Type and Single Pool Automation

Automatically triggered on exit of the Pooling screen, this automation checks the following information:

- All samples in the pool have the same Flowcell Type assigned to them.
- Only one pool has been created.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/novaseq/novaseq-
extensions.jar script:validate_flowcell_for_input_pools -i {stepURI:v2} -u
{username} -p {password} -l {compoundOutputFileLuid1} -validateSingleOutput true
-poolType bulk
```

Calculate Volumes Automation

Due to the 4000-character limit, part of the script definition of Calculate Volumes has been moved into the Calculate Volumes Script field. If this part of the script needs to be modified, you can make the changes to the default value of the Calculate Volumes Script field. The parts of the script that were moved into the Calculate Volumes Script field are identified below.

Automatically triggered when the Calculate Volumes button on the Record Details screen is selected, this automation does the following actions:

- Calculates the number of samples in the pool:

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: + 1
```

- Sets the value of the Bulk Pool Volume (µl) field, based on the selected Flowcell Type. The following part of the automation is found in the Calculate Volumes Script field:

```
step.::PhiX Volume (ul):: = !step.hasValue(::% PhiX (2.5nM) Spike-In::) ? 0 :
step.::% PhiX (2.5nM) Spike-In::;
if (input.::Flowcell Type:: == ::SP::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 100;
step.::PhiX Volume (ul):: *= 0.6
};
if (input.::Flowcell Type:: == ::S1::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 100;
step.::PhiX Volume (ul):: *= 0.6
};
if (input.::Flowcell Type:: == ::S2::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 150;
step.::PhiX Volume (ul):: *= 0.9
};
if (input.::Flowcell Type:: == ::S4::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 310;
step.::PhiX Volume (ul):: *= 1.9
};
if (step.::PhiX Volume (ul):: == 0) {
step.::PhiX Volume (ul):: = ::::
};
```


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- Calculates the Per Sample Volume (µl) to be added to the pool. The following part of the automation is found in the Calculate Volumes Script field:

```
input.::Per Sample Volume (µl):: = ( ( (input.::Final Loading Concentration
(pM):: * 5 / 1000) / input.::Normalized Molarity (nM):: ) * step.::Bulk Pool
Volume (µl):: ) / step.::Number of Samples in Pool::
```

> To ensure accurate pipetting of each sample in a pool for sequencing, the Per Sample Volume (µl) value must be equal to or higher than the Minimum Per Sample Volume (µl). The default value is set at 5, which can be edited.
>
> Assuming the default Minimum Per Sample Volume (µl) value of 5, for a given batch:
>
> 1. If the smallest Per Sample Volume (µl) value is less than 5, Clarity LIMS automatically assigns a value of 5 to the Adjusted Per Sample Volume (µl) field.
> 2. Clarity LIMS then adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

- Calculates the Total Sample Volume (µl) field value:

```
step.::Total Sample Volume (µl):: = step.::Total Sample Volume (µl):: +
input.::Adjusted Per Sample Volume (µl)::
```

- If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) field value:

```
if (step.::Total Sample Volume (µl):: >= step.::Bulk Pool Volume (µl)::)
{output.::RSB Volume (µl):: = 0} else {output.::RSB Volume (µl):: = step.::Bulk
Pool Volume (µl):: - step.::Total Sample Volume (µl)::} ;
```

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs:

```
output.::Flowcell Type::= input.::Flowcell Type::;output.::Loading Workflow
Type::= input.::Loading Workflow Type::;
```

- Sets the Volume of Pool to Denature (µl) value and calculates NaOH Volume (µl) and Tris-HCl Volume (µl) values, based on the Flowcell Type:

```
step.::PhiX Volume (ul):: = !step.hasValue('% PhiX (2.5nM) Spike-In') ? 0 :
step.'% PhiX (2.5nM) Spike-In';
if (input.::Flowcell Type:: == ::SP::) {
output.::Volume of Pool to Denature (ul):: = 100;
output.::NaOH Volume (ul):: = 25;
output.::Tris-HCl Volume (ul):: = 25;
}
if (input.::Flowcell Type:: == ::S1::) {
output.::Volume of Pool to Denature (ul):: = 100;
output.::NaOH Volume (ul):: = 25;
```


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```
output.::Tris-HCl Volume (ul):: = 25;
}
if (input.::Flowcell Type:: == ::S2::) {
output.::Volume of Pool to Denature (ul):: = 150;
output.::NaOH Volume (ul):: = 37;
output.::Tris-HCl Volume (ul):: = 38;
}
if (input.::Flowcell Type:: == ::S4::) {
output.::Volume of Pool to Denature (ul):: = 310;
output.::NaOH Volume (ul):: = 77;
output.::Tris-HCl Volume (ul):: = 78;
}
```

- Uses the `NovaSeq_Standard_Bulk_Pool1.csv`, `NovaSeq_Standard_Bulk_Pool2.csv`, and `NovaSeq_Standard_Bulk_Pool3.csv` template files to generate a single CSV file containing information about the pool and the samples it contains. The generated file is available for download on the Step Setup screen of the following step: Dilute and Denature (NovaSeq 6000 v2.3).

```
script:driver_file_generator \ -t
/opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Standard_Bulk_Pool1.csv \
-o 1.csv \ script:driver_file_generator \ -t
/opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Standard_Bulk_Pool2.csv \
-o 2.csv \ script:driver_file_generator \ -t
/opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Standard_Bulk_Pool3.csv \
-o 3.csv \&& cat 1.csv 2.csv 3.csv > {compoundOutputFileLuid0}.csv
```

- Resets the Total Sample Volume (ul) and Number of Samples in Pool field values so that the automation is idempotent:

```
step.'Number of Samples in Pool' = step.'Number of Samples in Pool' - 1;
step.'Total Sample Volume (ul)' = step.'Total Sample Volume (ul)' -
input.'Adjusted Per Sample Volume (ul)'
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, the `nextStep = ::ADVANCE::` automation sets the next step for samples to ADVANCE, advancing them to the Dilute and Denature (NovaSeq 6000 v2.3) step in the protocol.

Master Step Fields

The following table provides field configuration details for the fields defined on the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.3) step.

Table 243 Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.3) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Drop-Down Items
% PhiX (2.5nM) Spike-In	Numeric		Range = 1–100 Decimal places displayed = 0
Bulk Pool Volume (µl) (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 2
Minimum Per Sample Volume (uL)	Numeric	Required Field	Default • 5 Decimal places displayed = 2
Number of Flowcells to Sequence	Numeric	Required Field	Range = 1–10 Decimal places displayed = 0
Number of Samples in Pool (for calculation purposes, not displayed)	Numeric		Default • 0 Decimal places displayed = 0
PhiX Volume (µl) (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 2
Total Sample Volume (µl) (for calculation purposes, not displayed)	Numeric		Default • 0 Decimal places displayed = 2
Calculate Volumes Script	Text	Required Field Read Only	Default value provided in the drop-down section following the table. Do not remove this field. It is used by the Calculate Volumes automation script.

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Calculate Volumes Script

```
step.::PhiX Volume (ul):: = !step.hasValue(::% PhiX (2.5nM) Spike-In::) ? 0 :
step.::% PhiX (2.5nM) Spike-In::; if (input.::Flowcell Type:: == ::SP::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 100;
step.::PhiX Volume (ul):: *= 0.6 }; if (input.::Flowcell Type:: == ::S1::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 100;
step.::PhiX Volume (ul):: *= 0.6 }; if (input.::Flowcell Type:: == ::S2::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 150;
step.::PhiX Volume (ul):: *= 0.9 }; if (input.::Flowcell Type:: == ::S4::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 310;
step.::PhiX Volume (ul):: *= 1.9 }; if (step.::PhiX Volume (ul):: == 0) {
step.::PhiX Volume (ul):: = :::: }; input.::Per Sample Volume (ul):: =
(((input.::Final Loading Concentration (pM):: * 5 / 1000) / input.::Normalized
Molarity (nM)::) * step.::Bulk Pool Volume (ul)::) / step.::Number of Samples in
Pool::
```

Global Fields

The following table lists the global fields that are configured to display on the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.3) step.

Table 244 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Drop-Down Items
Configured on Derived Sample			
Flowcell Type	Text Dropdown	Required Field	Presets • S1 • S2 • S4 • SP
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
NaOH Volume (µl)	Numeric	Read Only	Decimal places displayed = 2

Field Name	Field Type	Options	Additional Options and Drop-Down Items
RSB Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Tris-HCl Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Volume of Pool to Denature (µl) (Used in Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.3) step only. Displays on Record Details screen and in the generated CSV file.)	Numeric	Read Only	Decimal places displayed = 0

User Interaction

1. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucket and select View Ice Bucket.
2. On the Ice Bucket screen, select Begin Work.
3. On the Pooling screen, create a pool as follows.
   a. Create a pool by dragging samples into the Pool Creator.
   b. Name the pool or accepts the default name (Pool #1).
   c. Select Record Detail.

   On exit from the Pooling screen, the Validate Inputs Flowcell Type and Single Pool is triggered. The automation checks that all samples in the pool have been assigned the same Flowcell Type and only one pool has been created.
4. On the Record Details screen in the Step Details area, complete the fields as follows.
   a. Enter the value for the Number of Flowcells to Sequence field.
      This value is used in volume calculations to make sure that the volumes are sufficient for the number of times the pool is sequenced.
   b. Enter the value for the Minimum Per Sample Volume (µl) field.
      The value in this field is used to calculate how much of each sample is included in the pool. The field is prepopulated with the configured default value (5 µl), but the value can be edited if necessary.
   c. If necessary, enter the value for the % PhiX (2.5 nM) Spike-In field.

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The value in this field is used to calculate the volume of PhiX v3 control to be included in the pool for the given percentage of spike-in. The field is optional and can be edited.

5. In the Sample Details table, select the pool icon to view details on the pool composition.
6. Select Calculate Volumes.
   This selection triggers the Calculate Volumes automation, which performs the following volume calculations based on the selected Flowcell Type:
   - Calculates Bulk Pool Volume (µl) value.
   - Calculates PhiX Volume (µl) value if % PhiX (2.5 nM) Spike-In has value.
   - Calculates the Per Sample Volume (µl) value to be added to the pool.
   - Calculates the Total Sample Volume (µl) value.
   - If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) value.
   - Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
   - Populates the Volume of Pool to Denature (µl), NaOH Volume (µl), and Tris-HCl Volume (µl) columns of the Sample Details table. (Values are set by a script and are not editable.)
   - Generates the Calculation File (CSV) and attaches it to the step. This file contains volume information about the pool and the individual samples it contains. Select the file to download it, then open it in Excel.
7. Select Next Steps.
   This selection triggers the Set Next Step automation. This automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol: Dilute and Denature (NovaSeq 6000 v2.3).
   On the Assign Next Steps screen, the next step is already set to Dilute and Denature (NovaSeq 6000 v2.3).
8. Select Finish Step.
   At the end of this step, the pool of samples automatically advances to the Dilute and Denature (NovaSeq 6000 v2.3) step.

Step 2: Dilute and Denature (NovaSeq 6000 v2.3)

In this step, pooled samples are denatured and diluted by the addition of NaOH, Tris-HCl, and Resuspension Buffer (RSB). Samples are manually placed into the library tube that is used in the NovaSeq run. In addition, this step generates the sample sheet file and the run recipe (*json file) needed to start the NovaSeq run. The step input is Bulk pool, and the step output is Library tube.

The following automations are configured on the step:

- Generate Sample Sheet and Run Recipe
- Prepare Files for NovaSeq
- Routing Script

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- Validate Library Tube Barcode
- Validate Single Input

Validate Single Input Automation

Automatically triggered at the beginning of the step, the `script:validateSampleCount -min 1 -max 1` automation checks that there is only one container input to the step.

Validate Library Tube Barcode Automation

Automatically triggered on exit of the Placement screen, the following automation validates the library tube barcode to make sure it conforms to the barcode mask [A-Z]{2}[0-9]{7}-[A-Z]{3}:

```
if (!output.container.name.matches(::[A-Z]{2}[0-9]{7}-[A-Z]{3}::)
fail(::Invalid Library Tube Barcode. Please verify and try again.::)
}
```

Generate Sample Sheet and Run Recipe Automation

Automatically triggered when a button on the Record Details screen is selected, this automation does the following actions:

- Copies the Flowcell Type from the step input to the step output:

```
output.::Flowcell Type:: = input.::Flowcell Type::
```

- Copies the Flowcell Type from the step input to the Run Mode field (hidden):

```
step.::Run Mode:: = input.::Flowcell Type::
```

- Copies the Loading Workflow Type values from the step inputs to the step outputs:

```
output.::Loading Workflow Type:: = input.::Loading Workflow Type::;
```

- Validates the parameters entered on the Record Details screen:
  - Experiment Name can only contain alphanumeric, dash, or underscore characters. Spaces are not permitted.
  - When Workflow Type is No Index, Index Read 1 must be zero. For any other Workflow Type, Index Read 1 must be greater than zero.

```
if(!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) {
fail(::Experiment Name contains prohibited characters. Allowed characters are:
a-z, A-Z, 0-9, -, and _::)
}
if(step.::Workflow Type::== ::No Index::) {
if(step.::Index Read 1::!= 0) {
fail(::Index Read 1 must be 0 if the Workflow Type is No Index.::)
}
} else {
```


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```
if(step.::Index Read 1::== 0) {
fail(::Index Read 1 must be greater than 0 if the Workflow Type is ::+
step.::Workflow Type::+ ::.::)
}
}
```

- When Paired End is True, Index Read 1 and Index Read 2 must be greater than zero.
- When Paired End is False, Index Read 2 must be zero, Reverse Complement Workflow must be false, UMI - Read 2 Length must not have a value, and UMI - Read 2 Start from Cycle must not have a value.

```
if (step.::Paired End::.toBoolean()) {
if (step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) {
fail(::Read 1 Cycles and Read 2 Cycles must be greater than 0 if Paired End is
True.::);
}
} else {
if (step.::Read 2 Cycles:: != 0) {
fail(::Read 2 Cycles must be 0 if Paired End is False.::);
}
if (step.hasValue(::UMI - Read 2 Length::) || step.hasValue(::UMI - Read 2 Start
From Cycle::)) {
fail(::UMI - Read 2 Length and UMI - Read 2 Start From Cycle cannot be defined
if Paired End is False.::);
}
}
```

- Validates allowed read cycles to be not greater than 151 if the Flowcell Type is not SP.

```
if (input.::Flowcell Type:: != ::SP:: && step.::Read 1 Cycles:: > 151) {
fail(::Read 1 Cycles must not be larger than 151 if it is not SPrime
Flowcell.::);
}
if (input.::Flowcell Type:: != ::SP:: && step.::Read 2 Cycles:: > 151) {
fail(::Read 2 Cycles must not be larger than 151 if it is not SPrime
Flowcell.::);
}
```

- Validates allowed value for UMI - Read 1 Length, UMI - Read 2 Length, UMI - Read 1 From Cycle, UMI - Read 2 From Cycle.

```
if (step.hasValue(::UMI - Read 1 Length::) && !step.hasValue(::UMI - Read 1
Start From Cycle::)) {
fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 1
Length is greater than 0.::);
```

```
}
if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 1
Start From Cycle::)) {
fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 1 Start From
Cycle is greater than 0.::);
}
if (step.hasValue(::UMI - Read 2 Length::) && !step.hasValue(::UMI - Read 2
Start From Cycle::)) {
fail(::UMI - Read 2 Start From Cycle must be greater than 0 if UMI - Read 2
Length is greater than 0.::);
}
if (!step.hasValue(::UMI - Read 2 Length::) && step.hasValue(::UMI - Read 2
Start From Cycle::)) {
fail(::UMI - Read 2 Length must be greater than 0 if UMI - Read 2 Start From
Cycle is greater than 0.::);
}
if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 2
Length::)) {
fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 2 Length is
greater than 0.::);
}
if (!step.hasValue(::UMI - Read 1 Start From Cycle::) && step.hasValue(::UMI -
Read 2 Start From Cycle::)) {
fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 2
Start From Cycle is greater than 0.::);
}
```

- Sets the next step for samples to REMOVE.

```
nextStep = ::REMOVE::
```

- Generates the sample sheet and the run recipe file and attaches them to the step. For details on how the files are generated and their contents, refer to *Sample Sheet and Run Recipe File Generation* on page 1056.

Prepare Files for NovaSeq Automation

Automatically triggered on exit of the Record Details screen, this automation invokes the `copy_attachments_to_network_folder` script, which is included in the `novaseq-remote-extensions.jar` file. This script places the generated sample sheet and run recipe (*json file) into the appropriate folder on the NAS, where the instrument uses them to start the run.

For more information, refer to *Sample Sheet and Run Recipe File Generation* on page 1056.

Routing Script Automation

Automatically triggered on exit of the step, this automation invokes the changeWorkflow script, which routes step outputs to the NovaSeq 6000 v2.3 workflow, and queues them for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) step.

The default automation command line is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -u {username} -p {password} -i {stepURI:v2} -l {compoundOutputFileLuid2} script:changeWorkflow \
\
--FIELD_NAME 'N/A' \
--FIELD_VALUE 'N/A' \
--WORKFLOW 'NovaSeq 6000 v2.3' \
--STEP 'AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3)' \
--INPUTS_OR_OUTPUTS 'OUTPUTS'"
```

Master Step Fields

The following table provides field configuration details fields defined on the Dilute and Denature (NovaSeq 6000 v2.3) step. These fields are required for sample sheet and JSON file generation.

Table 245 Dilute and Denature (NovaSeq 6000 v2.3) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Drop-Down Items
BaseSpace Sequence Hub Configuration	Text Dropdown	Required Field	Presets • Not Used • Run Monitoring Only • Run Monitoring and Storage
Experiment Name	Text	Required Field	
Index Read 1	Numeric Dropdown	Required Field Custom Entries	Range = 0–20 Decimal places displayed = 0 Presets • 0 • 6 • 8

Field Name	Field Type	Options	Additional Options and Drop-Down Items
Index Read 2	Numeric Dropdown	Required Field Custom Entries	Range = 0–20 Decimal places displayed = 0 Presets • 0 • 6 • 8
Paired End	Text Dropdown	Required Field	Presets • True • False
Read 1 Cycles	Numeric Dropdown	Required Field Custom Entries	Range = 1–251 Decimal places displayed = 0 Presets • 251* • 151 • 101 • 51 *The value of 251 is only supported for SP flow cell type. For all other flow cell types, the maximum value is 151.
Read 2 Cycles	Numeric Dropdown	Required Field Custom Entries	Range = 0–251 Decimal places displayed = 0 Presets • 251* • 151 • 101 • 51 *The value of 251 is only supported for SP flow cell type. For all other flow cell types, the maximum value is 151.

Field Name	Field Type	Options	Additional Options and Drop-Down Items
Reverse Complement Workflow	Toggle Switch	Required Field	Default • Yes
Run Mode (not displayed in user interface)	Text Dropdown	Read Only	Presets • SP • S1 • S2 • S4
Sample Sheet Path	Text	Read Only	
UMI - Read 1 Length	Numeric		Range = 1 Decimal places displayed = 0
UMI - Read 1 Start From Cycle	Numeric		Range = 1 Decimal places displayed = 0
UMI - Read 2 Length	Numeric		Range = 1 Decimal places displayed = 0
UMI - Read 2 Start From Cycle	Numeric		Range = 1 Decimal places displayed = 0
Use Custom Index Read 1 Primer	Toggle Switch		Default • No
Use Custom Read 1 Primer	Toggle Switch		Default • No
Use Custom Read 2 Primer	Toggle Switch		Default • No
Workflow	Text	Read Only	Default • GenerateFASTQ
Workflow Type	Text Dropdown	Required Field	Presets • No Index • Single Index • Dual Index

Global Step Fields

The following table lists the global step fields that are configured to display on the Dilute and Denature (NovaSeq 6000 v2.3) step.

Table 246 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Drop-Down Items
Configured on Derived Sample			
Flowcell Type	Text Dropdown	Required Field	Presets • S1 • S2 • S4 • SP
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User interaction

1. On the Placement screen, move the pool as follows.
   a. Drag the pool into the library tube in the Placed Samples area.
   b. Scan or types the barcode of the library tube into the Library Tube field.
   c. Select Record Details.
   On exit of the Placement screen, the Validate Library Tube Barcode automation checks that the library tube barcode conforms to the barcode mask [A-Z]{2}[0-9]{7}-[A-Z]{3}. If not, an error message displays.
2. On the Record Details screen:
   - The Reagent Lot Tracking section tracks the NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. Select from the active lots displayed in each drop-down list.

     To make reagent lots available for selection in the Reagent Lot Tracking section, configure and activate them. Do this step on the Configuration > Consumables screen.

   - The fields displayed in the Step Details section are used to generate the sample sheet and run recipe files. Some of these fields are autopopulated and some must be completed manually (refer to the following table for details).

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Table 247 Fields Displayed on Record Details Screen of Dilute and Denature (NovaSeq 6000 v2.3) Step

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.
BaseSpace Sequence Hub Configuration	Presets • Not Used • Run Monitoring Only • Run Monitoring and Storage
Workflow	Presets • GenerateFASTQ
Workflow Type	Presets • No Index • Single Index • Dual Index
Index Read 1	Presets • 0 • 6 • 8 • type a value between 0 and 20
Index Read 2	Presets • 0 • 6 • 8 • type a value between 0 and 20
Paired End	Presets • True • False
Read 1 Cycles	Presets • 251* • 151 • 101 • 51 • Type a value between 1 and 251 for the SP flow cell type or between 1 and 151 for all other types

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Field	Value
Read 2 Cycles	Presets • 251* • 151 • 101 • 51 • Type a value between 0 and 251 for the SP flow cell type or between 0 and 151 for all other types
Use Custom Read 1 Primer	Select if applicable.
Use Custom Read 2 Primer	Select if applicable.
Use Custom Index Read 1 Primer	Select if applicable.
Reverse Complement Workflow	Select Yes (by default) for v1.5 reagents in NovaSeq Control Software v1.7 and onwards. Select No for v1 reagents.
UMI - Read 1 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 1. Leave blank if not applicable.
UMI - Read 2 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 2. Leave blank if not applicable.
UMI - Read 1 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.
UMI - Read 2 Start From Cycle	Enter the cycle number that Read 2 starts from. Leave blank if not applicable.
Sample Sheet Path	Automatically populated after the sample sheet has been generated.

3. Select Generate Sample Sheet and Run Recipe.
   This selection triggers the automation script, which does the following actions:
   - Generates the sample sheet and JSON files and attaches them to the placeholders in the Files area of the Record Details screen.
   - Populates the Sample Sheet Path field with the path to the sample sheet file.
   - Sets the value of the next step to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step successfully.
4. Select Next Steps.
   This selection triggers the Prepare Files for NovaSeq automation, which copies the sample sheet and run recipe files to the location specified during installation.

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5. On the Assign Next Steps screen, make sure the Next Step for all samples is prepopulated with Remove from workflow.

   Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.

6. Select Finish Step.
   This will trigger the Routing Script automation and route samples to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) protocol. In Lab View, the pool of samples is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) step.

At this point in the workflow, the user interaction ends. The integration automatically starts the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) step and data from the run is parsed back into Clarity LIMS.

Protocol 3: NovaSeq Xp (NovaSeq 6000 v2.3)

Samples are routed to this protocol if the Loading Workflow Type value is set to NovaSeq Xp.

Samples are pooled and added to lanes on the NovaSeq flow cell type selected in the Define Run Format (NovaSeq 6000 v2.3) step.

At the end of the protocol, a routing script sends the flow cell to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) protocol.

This protocol contains three steps:

1. Step 1: Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.3)
2. Step 2: Dilute, Denature, and ExAmp (NovaSeq 6000 v2.3)
3. Step 3: Load to Flowcell (NovaSeq 6000 v2.3)

Step 1: Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.3)

In this step, the libraries are manually placed into a pool. The step input is NTP (normalized libraries) and the step output is Bulk pool.

Only create one pool per step.

The following automation is configured on the step:

- Calculate Volumes
- Set Next Step
- Validate Inputs Flowcell Type and Single Pool
- Validate Unique Indexes (Not used. This functionality is handled by the core Clarity LIMS configuration for pooling.)

These automations are described in the order in which they are triggered at run time.

Validate Inputs Flowcell Type and Single Pool

Automatically triggered on exit of the Pooling screen, this automation checks the following information:

- All samples in the pool have the same Flowcell Type assigned to them.
- Only one pool has been created.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/novaseq/novaseq-extensions.jar script:validate_flowcell_for_input_pools -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -validateSingleOutput true
```

Calculate Volumes

Automatically triggered when the Calculate Volumes button on the Record Details screen is selected, this automation:

- Calculates the number of samples in the pool:

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: + 1
```

- Sets the value of the Bulk Pool Volume (µl) and PhiX Volume (µl) field, based on the selected Flowcell Type:

```
step.::PhiX Volume (µl):: = !step.hasValue(::% PhiX (0.25nM) Spike-In::) ? 0 : step.::% PhiX (0.25nM) Spike-In::;
if (input.::Flowcell Type:: == ::SP::) {
step.::Bulk Pool Volume (µl):: = step.'Number of Lanes to Sequence' * 18;
step.::PhiX Volume (µl):: *= 0.7;
}
if (input.::Flowcell Type:: == ::S1::) {
step.::Bulk Pool Volume (µl):: = step.'Number of Lanes to Sequence' * 18;
step.::PhiX Volume (µl):: *= 0.7;
}
if (input.::Flowcell Type:: == ::S2::) {
step.::Bulk Pool Volume (µl):: = step.'Number of Lanes to Sequence' * 22;
step.::PhiX Volume (µl):: *= 0.8;
}
if (input.::Flowcell Type:: == ::S4::) {
step.::Bulk Pool Volume (µl):: = step.'Number of Lanes to Sequence' * 30;
step.::PhiX Volume (µl):: *= 1.1;
}
if (step.::PhiX Volume (µl):: == 0) {
step.::PhiX Volume (µl):: = '';
}
```

- Calculates the Per Sample Volume (µl) to be added to the pool:

```
input.::Per Sample Volume (µl)::= (((input.::Final Loading Concentration (pM)::*
5/ 1000) / input.::Normalized Molarity (nM)::) * step.::Bulk Pool Volume (µl)::)
/ step.::Number of Samples in Pool::
```

> To ensure accurate pipetting of each sample in a pool for sequencing, the Per Sample Volume (µl) value must be equal to or higher than the Minimum Per Sample Volume (µl). The default value set at 5 and can be edited.
>
> Assuming the default Minimum Per Sample Volume (µl) value of 5, for a given batch:
>
> 1. If the smallest Per Sample Volume (µl) value is less than 5, Clarity LIMS automatically assigns a value of 5 to the sample's Adjusted Per Sample Volume (µl) field.
> 2. Clarity LIMS then adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

- Calculates the Total Sample Volume (µl) field value:

```
step.::Total Sample Volume (µl):: = step.::Total Sample Volume (µl):: +
input.::Adjusted Per Sample Volume (µl)::
```

- If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) field value:

```
if (step.::Total Sample Volume (µl):: >= step.::Bulk Pool Volume (µl)::)
{output.::RSB Volume (µl):: = 0} else {output.::RSB Volume (µl):: = step.::Bulk
Pool Volume (µl):: - step.::Total Sample Volume (µl)::} ;
```

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs:

```
output.::Flowcell Type::= input.::Flowcell Type::;output.::Loading Workflow
Type::= input.::Loading Workflow Type::;
```

- Uses the `NovaSeq_Xp_Bulk_Pool.csv` and `NovaSeq_Xp_Bulk_Pool2.csv` template files to generate a single CSV file containing information about the bulk pool and the samples it contains. The generated file is available for download on the Step Setup screen of the following step—Dilute, Denature, and ExAmp (NovaSeq 6000 v2.3).
- Resets the Total Sample Volume (ul) and Number of Samples in Pool field values so that the automation is idempotent:

```
step.'Number of Samples in Pool' = step.'Number of Samples in Pool' - 1;
step.'Total Sample Volume (ul)' = step.'Total Sample Volume (ul)' -
input.'Adjusted Per Sample Volume (ul)'
```

Set Next Step

Automatically triggered on exit of the Record Details screen, this automation does the following actions:

- Copies the Flowcell Type values from the step inputs to the step outputs.

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- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ; nextStep = ::ADVANCE::
```

Master Step Fields

The following table provides field configuration details for the fields defined on the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.3) step.

Table 248 Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.3) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Drop-Down Items
% PhiX (0.25nM) Spike-In	Numeric		Range = 1–100 Decimal places displayed = 0
Bulk Pool Volume (µl) (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 2
Minimum Per Sample Volume (µl)	Numeric	Required Field	Default • 5 Decimal places displayed = 2
Number of Lanes to Sequence	Numeric	Required Field	Decimal places displayed = 0
Number of Sample in Pool (for calculation purposes, not displayed)	Numeric		Default • 0 Decimal places displayed = 0
PhiX Volume (µl) (for calculation purposes, not displayed)	Numeric		Decimal places displayed = 2
Total Sample Volume (µl) (for calculation purposes, not displayed)	Numeric		Default • 0 Decimal places displayed = 0

Global Fields

The following table lists the global fields that are configured to display on the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.3) step.

Table 249 Global Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Configured on Derived Sample			
RSB Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Flowcell Type	Text Dropdown	Required Field	Presets • S1 • S2 • S4 • SP
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User Interaction

1. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucket and select View Ice Bucket.
2. On the Ice Bucket screen, select Begin Work.
3. On the Pooling screen, create a pool as follows.
   a. Drag samples into the Pool Creator to create a pool.
   b. Name the pool or accepts the default name Pool #1.
   c. Select Record Details.

   On exit from the Pooling screen, the Validate Inputs Flowcell Type and Single Pool is triggered. The automation checks that all samples in the pool have been assigned the same Flowcell Type and only one pool has been created.
4. On the Record Details screen in the Step Details area, complete the fields as follows.
   a. Enter the value for the Number of Lanes to Sequence field.
      This value is used in volume calculations to make sure that the volumes are sufficient for the number of times the pool is sequenced.
   b. Enter the value for the Minimum Per Sample Volume (µl) field.

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The value in this field is used to calculate how much of each sample is included in the pool. The field is prepopulated with the configured default value (5 µl), but the value can be edited if necessary.

c. If necessary, enter the value for the % PhiX (0.25 nM) Spike-In field.
The value in this field is used to calculate the volume of PhiX v3 control to be included in the pool for the given percentage of spike-in. The field is optional and can be edited.

5. In the Sample Details table, select the pool icon to view details on the pool composition.
6. Select Calculate Volumes.
This selection triggers the Calculate Volumes automation, which performs the following volume calculations based on the selected Flowcell Type:
   - Calculates Bulk Pool Volume (µl) value.
   - If % PhiX (0.25nM) Spike-In has a value, calculates the PhiX Volume (µl) value.
   - Calculates the Per Sample Volume (µl) value to be added to the pool.
   - Calculates the Total Sample Volume (µl) value.
   - If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) value.
   - Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
   - Generates the Calculation File (*csv) and attaches it to the step. This file contains volume information about the samples and RSB buffer to add to the pool. Select the file to download it, then open it in Excel.
7. Select Next Steps.
This selection triggers the Set Next Step automation. This automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol—Dilute, Denature, and ExAmp (NovaSeq 6000 v2.3).
On the Assign Next Steps screen, the next step is already set to Dilute, Denature, and ExAmp (NovaSeq 6000 v2.3).
8. Select Finish Step.
At the end of this step, the pool of samples automatically advances to the Dilute, Denature, and ExAmp (NovaSeq 6000 v2.3) step.

Step 2: Dilute, Denature, and ExAmp (NovaSeq 6000 v2.3)

In this step, pooled samples are denatured and diluted by the addition of DPX, NaOH, Tris-HCl, and RSB. Manually create working pools based on the number of lanes you want to sequence. The step input is Bulk pool and the step output is Working pool. Working pool is a variable number for how many working pools need to be created per bulk pool.

The following automations are configured on the step:

- Calculate Volumes

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- Set Next Step
- Validate Inputs Flowcell Type

These automations are described in the order in which they are triggered at run time.

Validate Inputs Flowcell Type Automation

Automatically triggered at the beginning of the step, this automation invokes the `validate_flowcell_for_input_pools` script. This script does the following actions:

- Checks the inputs to the step and validates that the Flowcell Type field has been set to a valid value (SP, S1, S2, or S4). The automation also checks that all inputs have the same value.
- Validates that the number of outputs matches the number of lanes on the selected flow cell type. If validation fails, an error message state that the number of working pools does not match the number of lanes available on the flow cell.

```
bash -l -c "/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/novaseq/novaseq-extensions.jar script:validate_
flowcell_for_input_pools -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1}"
```

Calculate Volumes Automation

Automatically triggered on entry to of the Record Details screen, this automation sets the value of the following fields, based on the Flowcell Type:

- BP Aliquot Volume (µl)
- NaOH Volume (µl)
- Tris-HCl Volume (µl)
- DPX1 Volume (µl)
- DPX2 Volume (µl)
- DPX3 Volume (µl)
- Mastermix per Lane (µl)

```
if (input.::Flowcell Type:: == ::SP::) {
output.::BP Aliquot Volume (ul):: = 18;
output.::NaOH Volume (ul):: = 4;
output.::Tris-HCl Volume (ul):: = 5;
step.::DPX1 Volume (ul):: = 126;
step.::DPX2 Volume (ul):: = 18;
step.::DPX3 Volume (ul):: = 66;
output.::Mastermix per Lane (ul):: = 63
};
```


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```
if (input.::Flowcell Type:: == ::S1::) {
output.::bp Aliquot Volume (µl):: = 18;
output.::NaOH Volume (µl):: = 4;
output.::Tris-HCl Volume (µl):: = 5;
step.::DPX1 Volume (µl):: = 126;
step.::DPX2 Volume (µl):: = 18;
step.::DPX3 Volume (µl):: = 66;
output.::Mastermix per Lane (µl):: = 63
};
if (input.::Flowcell Type:: == ::S2::) {
output.::bp Aliquot Volume (µl):: = 22;
output.::NaOH Volume (µl):: = 5;
output.::Tris-HCl Volume (µl):: = 6;
step.::DPX1 Volume (µl):: = 126;
step.::DPX2 Volume (µl):: = 18;
step.::DPX3 Volume (µl):: = 66;
output.::Mastermix per Lane (µl):: = 77
};
if (input.::Flowcell Type:: == ::S4::) {
output.::bp Aliquot Volume (µl):: = 30;
output.::NaOH Volume (µl):: = 7;
output.::Tris-HCl Volume (µl):: = 8;
step.::DPX1 Volume (µl):: = 315;
step.::DPX2 Volume (µl):: = 45;
step.::DPX3 Volume (µl):: = 165;
output.::Mastermix per Lane (µl):: = 105
};
```

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs:

```
output.::Flowcell Type::= input.::Flowcell Type::;
output.::Loading Workflow Type::= input.::Loading Workflow Type::;
```

- Uses the `NovaSeq_Xp_Working_Pool.csv` and `NovaSeq_Xp_Working_Pool2.csv` template files to generate a single CSV file containing information about the DPX volume and the volume of bp Aliquot, Mastermix, NaOH, and Tris-HCl to add per working pool. The generated file is available for download on the Step Setup screen of the following step—Load to Flowcell (NovaSeq 6000 v2.3).

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation:

- Copies the Flowcell Type values from the step inputs to the step outputs.
- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ; nextStep = ::ADVANCE::
```

Master Step Fields

The following table provides field configuration details for the fields defined on Dilute, Denature, and ExAmp (NovaSeq 6000 v2.3) step. These field values are set by a script and are not editable while running the step.

Table 250 Dilute, Denature, and ExAmp (NovaSeq 6000 v2.3) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Drop-Down Items
DPX1 Volume (µl)	Numeric	Read Only	Decimal places displayed = 0
DPX2 Volume (µl)	Numeric	Read Only	Decimal places displayed = 0
DPX3 Volume (µl)	Numeric	Read Only	Decimal places displayed = 0

Global Fields

The following table lists the global fields that are configured to display on the Dilute, Denature, and ExAmp (NovaSeq 6000 v2.3) step.

Table 251 Global Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Configured on Derived Sample			
BP Aliquot Volume (µl)	Numeric	Read Only	Decimal places displayed = 0
Flowcell Type	Text Dropdown	Required Field	Presets • SP • S1 • S2 • S4

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Loading Workflow Type	Text Dropdown	Required Field	Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
Mastermix per Lane (µl)	Numeric	Read Only	Decimal places displayed = 0
NaOH Volume (µl)	Numeric	Read Only	Decimal places displayed = 2
Tris-HCl Volume (µl)	Numeric	Read Only	Decimal places displayed = 2

User Interaction

1. On the Queue screen, add the pool to the Ice Bucket and select View Ice Bucket.
2. On the Ice Bucket screen, set the number of derivatives to create (they are placed into the flow cell lanes) and select Begin Work.
   On entry to the Record Details screen, the Calculate Volumes automation is triggered.
   This automation sets the following values based on the selected Flowcell Type (calculation details are provided in the previous Calculate Volumes Automation section):
   - BP Aliquot Volume (µl)
   - NaOH Volume (µl)
   - Tris-HCl Volume (µl)
   - DPX1 Volume (µl)
   - DPX2 Volume (µl)
   - DPX3 Volume (µl)
   - Mastermix per Lane (µl)

   The automation also generates the Calculation File (*csv) and attaches it to the step. This file contains information about the DPX Mastermix volume and the volume of Mastermix, NaOH, and Tris-HCl to add per working pool. Select the file to download it, then open it in Excel.
3. On the Record Details screen, the Reagent Lot Tracking section tracks the DPX1, DPX2, DPX3, NaOH, RSB, and Tris-HCl reagent lots. Select from the active lots displayed in each drop-down list.

> To make reagent lots available for selection in the Reagent Lot Tracking section, configure and activate them. Do this step on the Configuration > Consumables screen.

4. In the Step Details area, the DPX1, DPX2, and DPX3 reagent volume values are already populated. (These values are set by a script and are not editable while running the step.)
5. In the Sample Details table, select the pool icon to view details on the working pool composition.
   - The BP Aliquot, Mastermix per lane, NaOH, and Tris-HCl volume values for each working pool are already populated. (These values are set by a script and are not editable while running the step.)
   - The working pool number is appended to the bulk pool name. This feature helps quickly identify which working pools are derived from the same bulk pool.
6. In the Files area, select the Calculation File (*csv) file to download it. Open the file in Excel to view information about the DPX Mastermix volume and the volume of Mastermix, NaOH, and Tris-HCl to add per working pool.
7. Select Next Steps to trigger the Set Next Step automation.
   On the Assign Next Steps screen, the next step is already set to Load to Flowcell (NovaSeq 6000 v2.3).
8. Select Finish Step.

Step 3: Load to Flowcell (NovaSeq 6000 v2.3)

In this step, scan the flow cell barcode into Clarity LIMS, then manually place the working pools into the lanes of the flow cell that is used in the NovaSeq run. The step input is Working pool and the step output is Flow cell (output containers: S1 and S2 with 2 lanes, and S4 with 4 lanes).

The following automations are configured on this step:

- Generate Sample Sheet and Run Recipe
- Prepare Files for NovaSeq
- Validate Flowcell Barcode
- Validate Inputs and Selected Container

These automations are described in the order in which they are triggered at run time.

Validate Inputs and Selected Container Automation

Automatically triggered at the beginning of the step, this automation invokes the `validate_flowcell_for_input_pools` and `validateSelectedContainer` scripts. These scripts validate the step inputs and the container selected, as follows.

- Check that the Flowcell Type field has been set to a valid value (S1, S2, S4, or SP) and that all inputs have the same value.

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- Check that the number of outputs matches the number of lanes on the selected flow cell type. If validation fails, an error message states that the number of working pools does not match the number of lanes available on the flow cell. The following table defines the number of samples allowed:

Flowcell Type	Number of Working Pools
SP	2
S1	2
S2	2
S4	4

- Check that the container type selected on entry to the Placement screen matches the value in the Flowcell Type field. If validation fails, an error message displays.

Validate Flowcell Barcode

Automatically triggered on exit of the Placement screen, this automation validates the flow cell barcode scanned into Clarity LIMS, using the following logic:

```
if (input.::Flowcell Type:: == ::SP:: && !output.container.name.matches(::[A-Z0-9]{5}DR[A-Z0-9]{2}::)) {
fail(::Invalid Flowcell Barcode. Please verify and try again.::)
};
if (input.::Flowcell Type:: == ::S1:: && !output.container.name.matches(::[A-Z0-9]{5}DR[A-Z0-9]{2}::)) {
fail(::Invalid Flowcell Barcode. Please verify and try again.::)
};
if (input.::Flowcell Type:: == ::S2:: && !output.container.name.matches(::[A-Z0-9]{5}DM[A-Z0-9]{2}::)) {
fail(::Invalid Flowcell Barcode. Please verify and try again.::)
};
if (input.::Flowcell Type:: == ::S4:: && !output.container.name.matches(::[A-Z0-9]{5}DS[A-Z0-9]{2}::)) {
fail(::Invalid Flowcell Barcode. Please verify and try again.::)
};
```

Generate Sample Sheet and Run Recipe

Automatically triggered when a button on the Record Details screen is selected, this automation:

- Copies the Flowcell Type from the step input to the Run Mode field (hidden):

```
step.::Run Mode:: = input.::Flowcell Type::
```

- Validates the parameters entered on the Record Details screen:
  - Experiment Name can only contain alphanumeric, dash, or underscore characters. Spaces are not permitted.
  - When Workflow Type is No Index, Index Read 1 must be zero. For any other Workflow Type, Index Read 1 must be greater than zero.

```
if(!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) {
fail(::Experiment Name contains prohibited characters. Allowed characters are:
a-z, A-Z, 0-9, -, and _::)
};
if(step.::Workflow Type::== ::No Index::) {
if(step.::Index Read 1::!= 0) {
fail(::Index Read 1 must be 0 if the Workflow Type is No Index.::)
}
} else{
if(step.::Index Read 1::== 0) {
fail(::Index Read 1 must be greater than 0 if the Workflow Type is ::+
step.::Workflow Type::+ ::.::)
}
};
```

  - When Paired End is True, Index Read 1 and Index Read 2 must be greater than zero.
  - When Paired End = False, Index Read 2 must be zero. Reverse Complement Workflow must be false. UMI - Read 2 Length and UMI - Read 2 Start from Cycle must not have a value.

```
if (step.::Paired End::.toBoolean()) {if (step.::Read 1 Cycles:: == 0 ||
step.::Read 2 Cycles:: == 0) {fail(::Read 1 Cycles and Read 2 Cycles must be
greater than 0 if Paired End is True.::);}} else {if (step.::Read 2 Cycles:: !=
0) {fail(::Read 2 Cycles must be 0 if Paired End is False.::);}
if (step.hasValue(::UMI - Read 2 Length::) || step.hasValue(::UMI - Read 2 Start
From Cycle::)) {fail(::UMI - Read 2 Length and UMI - Read 2 Start From Cycle
cannot be defined if Paired End is False.::);}}
```

  - Validates allowed read cycles to be not greater than 151 if the Flowcell Type is not SP.

```
if (input.::Flowcell Type:: != ::SP:: && step.::Read 1 Cycles:: > 151) {
fail(::Read 1 Cycles must not be larger than 151 if it is not SPrime
Flowcell.::);
}
if (input.::Flowcell Type:: != ::SP:: && step.::Read 2 Cycles:: > 151) {
fail(::Read 2 Cycles must not be larger than 151 if it is not SPrime
Flowcell.::);
}
```

- Validates allowed value for UMI - Read 1 Length, UMI - Read 2 Length, UMI - Read 1 From Cycle, UMI - Read 2 From Cycle.

```
if (step.hasValue(::UMI - Read 1 Length::) && !step.hasValue(::UMI - Read 1
Start From Cycle::)) {
fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 1
Length is greater than 0.::);
}
if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 1
Start From Cycle::)) {
fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 1 Start From
Cycle is greater than 0.::);
}
if (step.hasValue(::UMI - Read 2 Length::) && !step.hasValue(::UMI - Read 2
Start From Cycle::)) {
fail(::UMI - Read 2 Start From Cycle must be greater than 0 if UMI - Read 2
Length is greater than 0.::);
}
if (!step.hasValue(::UMI - Read 2 Length::) && step.hasValue(::UMI - Read 2
Start From Cycle::)) {
fail(::UMI - Read 2 Length must be greater than 0 if UMI - Read 2 Start From
Cycle is greater than 0.::);
}
if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 2
Length::)) {
fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 2 Length is
greater than 0.::);
}
if (!step.hasValue(::UMI - Read 1 Start From Cycle::) && step.hasValue(::UMI -
Read 2 Start From Cycle::)) {
fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 2
Start From Cycle is greater than 0.::);
}
```

- Copies the Flowcell Type value from the step inputs to the step outputs:

```
output.::Flowcell Type::= input.::Flowcell Type::;
```

- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol—AUTOMATED - Run (NovaSeq 6000) v2.3.

```
nextStep = ::ADVANCE::
```

- Generates the sample sheet and the run recipe file and attaches them to the step. For details on how the files are generated, and their contents, refer to *Sample Sheet and Run Recipe File Generation* on page 1056.

Prepare Files for NovaSeq

Automatically triggered on exit of the Record Details screen, this automation invokes the `copy_attachments_to_network_folder` script, which is included in the `novaseq-remote-extensions.jar` file. This script places the generated sample sheet and run recipe (*json file) into the appropriate folder on the NAS, where the instrument uses them to start the run.

For more information, refer to *Sample Sheet and Run Recipe File Generation* on page 1056.

Master Step Fields

The following table provides field configuration details for the fields defined on the Load to Flowcell (NovaSeq 6000 v2.3) step.

Table 252 Load to Flowcell (NovaSeq 6000 v2.3) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Drop-Down Items
BaseSpace Sequence Hub Configuration	Text Dropdown	Required Field	Presets • Not Used • Run Monitoring Only • Run Monitoring and Storage
Experiment Name	Text	Required Field	
Index Read 1	Numeric Dropdown	Required Field Custom Entries	Range = 0–20 Decimal places displayed = 0 Presets • 0 • 6 • 8
Index Read 2	Numeric Dropdown	Required Field Custom Entries	Range = 0–20 Decimal places displayed = 0 Presets • 0 • 6 • 8
Library Tube Barcode	Text	Required Field	

Field Name	Field Type	Options	Additional Options and Drop-Down Items
Paired End	Text Dropdown	Required Field	Presets • True • False
Read 1 Cycles	Numeric Dropdown	Required Field Custom Entries	Range = 1–251 Decimal places displayed = 0 Presets • 251* • 151 • 101 • 51 *The value of 251 is only supported for SP flow cell type. For all other flow cell types, the maximum value is 151.
Read 2 Cycles	Numeric Dropdown	Required Field Custom Entries	Range = 0–251 Decimal places displayed = 0 Presets • 251* • 151 • 101 • 51 *The value of 251 is only supported for SP flow cell type. For all other flow cell types, the maximum value is 151.
Reverse Complement Workflow	Toggle Switch	Required Field	Default • Yes

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Field Name	Field Type	Options	Additional Options and Drop-Down Items
Run Mode (not displayed in user interface)	Text Dropdown	Read Only	Presets • SP • S1 • S2 • S4
Sample Sheet Path	Text	Read Only	
UMI - Read 1 Length	Numeric		Range = 1 Decimal places displayed = 0
UMI - Read 1 Start From Cycle	Numeric		Range = 1 Decimal places displayed = 0
UMI - Read 2 Length	Numeric		Range = 1 Decimal places displayed = 0
UMI - Read 2 Start From Cycle	Numeric		Range = 1 Decimal places displayed = 0
Use Custom Index Read 1 Primer	Toggle Switch		Default • No
Use Custom Read 1 Primer	Toggle Switch		Default • No
Use Custom Read 2 Primer	Toggle Switch		Default • No
Workflow	Text	Read Only	Default • GenerateFASTQ
Workflow Type	Text Dropdown	Required Field	Presets • No Index • Single Index • Dual Index

Global Fields

The following table lists the global fields that are configured to display on the Load to Flowcell (NovaSeq 6000 v2.3) step.

Field Name	Field Type	Options	Additional Options and Drop-Down Items
Configured on Derived Sample			
Flowcell Type	Text Dropdown	Required Field	Presets • S1 • S2 • S4 • SP

User Interaction

1. On the Ice Bucket screen, select the appropriate flow cell type from the Destination Container drop-down list in the Container Options panel.
2. Select Begin Work.
3. On the Placement screen, move the pools as follows.
   a. Drag the pools from the left of the screen over into the Placed Samples area on the right.
   b. Scan or type the barcode of the flow cell into the flow cell field.
   c. Select Record Details.

   On exit of the Placement screen, the Validate Flowcell Barcode automation validates the container barcode (described previously). The fields displayed on the Record Details screen are used to generate the sample sheet and run recipe files. Some of these fields are autopopulated and some must be completed manually.

Table 253 Fields Displayed on Record Details Screen of Load to Flowcell (NovaSeq 6000 v2.3) Step

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.
BaseSpace Sequence Hub Configuration	Presets • Not Used • Run Monitoring Only • Run Monitoring and Storage
Workflow	Presets • GenerateFASTQ

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Field	Value
Workflow Type	Presets • No Index • Single Index • Dual Index
Index Read 1	Presets • 0 • 6 • 8 • Type a value between 0 and 20
Index Read 2	Presets • 0 • 6 • 8 • Type a value between 0 and 20
Paired End	Presets • True • False
Read 1 Cycles	Presets • 251 • 151 • 101 • 51 • Type a value between 1 and 251 for the SP flow cell type or between 1 and 151 for all other types
Read 2 Cycles	Presets • 251 • 151 • 101 • 51 • Type a value between 0 and 251 for the SP flow cell type or between 0 and 151 for all other types
Use Custom Read 1 Primer	Select if applicable.
Use Custom Read 2 Primer	Select if applicable.
Use Custom Index Read 1 Primer	Select if applicable.

For Research Use Only. Not for use in diagnostic procedures.

Field	Value
Reverse Complement Workflow	Select Yes (by default) for v1.5 reagents in NovaSeq Control Software v1.7 and onwards. Select No for v1 reagents.
UMI - Read 1 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 1. Leave blank if not applicable.
UMI - Read 2 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 2. Leave blank if not applicable.
UMI - Read 1 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.
UMI - Read 2 Start From Cycle	Enter the cycle number that Read 2 starts from. Leave blank if not applicable.
Sample Sheet Path	Automatically populated after the sample sheet has been generated.
Library Tube Barcode	Scan the library tube barcode.

4. On the Record Details screen, select Generate Sample Sheet and Run Recipe.
   This selection triggers the automation script, which does the following actions:
   - Generates the sample sheet and JSON files and attaches them to the placeholders in the Files area of the Record Details screen.
   - Populates the Sample Sheet Path field with the path to the sample sheet file.
5. Select Next Steps to trigger the Prepare Files for NovaSeq automation, which copies the sample sheet and run recipe files to the location specified during installation.
   On the Assign Next Steps screen, the Next Step for all samples is prepopulated with Mark protocol as complete.
6. Select Finish Step.
   In Lab View, the pool of samples is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) step.

At this point in the workflow, the user interaction ends. The integration automatically starts the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) step and data from the run is parsed back into Clarity LIMS.

Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3)

This final protocol contains one fully automated step, AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3).

Step 1: AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3)

Step input: Library tube from the NovaSeq Standard protocol or flow cell from the NovaSeq Xp protocol.

Step output: Result file and measurement.

> Do not start this step. This is a fully automated step. The sequencing service does not update samples correctly if they have been manually started.

In this step, pooled samples are sequenced on the NovaSeq instrument. The run data network folder is monitored by the sequencing service and automation worker to determine when the sequencing run begins and ends.

1. When a run is initiated on the NovaSeq instrument, the NovaSeq Control Software (NVCS) looks for the sample sheet and run recipe (*.json file) on the shared network drive.
   - The NVCS copies the sample sheet to the run directory.
   - The NVCS uses the run recipe to start the sequencing run.
2. When the run starts:
   - The instrument software creates a new run folder (named < libraryID >) on the shared network drive and copies the following files into that folder:
     - RunParameters.xml
     - RunInfo.xml
     - LIMS/ <libraryID>.json file (run recipe)
   - The Real-Time Analysis v3 (RTA3) software creates the InterOp folder.
3. The sequencing service detects the presence of the RunParameters, RunInfo, and JSON files and starts the AUTOMATED- NovaSeq Run (NovaSeq 6000 v2.3) step in Clarity LIMS.
4. As the run progresses, the InterOp data files are filled in.
5. At the end of the run, the NovaSeq:
   - Copies the run data files to the InterOp folder.
   - Creates the `CopyComplete.txt` file in the run folder. This step indicates to the sequencing service that the run has completed and the data files are ready.
6. The sequencing service triggers the Read InterOp Metrics automation. This automation reads the files, records the parsed metrics into Clarity LIMS, and finally completes the step in Clarity LIMS.

Parse Run Parameters and Generate Link to Run Folder Automation

Automatically triggered on entry to the Record Details screen, this automation does the following actions:

- Parses the `RunParameters.xml` file attached on the Step Setup screen.
- Populates the master and global step fields with extracted values.

- If there is an instrument in Clarity LIMS that matches the instrument name in the file (InstrumentName field), the script associates this instrument with the run.
- Generates and attaches a link to the run folder using the run output folder parsed from `RunParameters.xml`.

The following rules and constraints are present:

- If there is no InstrumentName value in the file, the script continues, but it logs a warning.
- If there is an InstrumentName value in the file and multiple instruments with the same name exist in Clarity LIMS, the script fails and logs an error.
- If the `RunParameters.xml` file is not attached to the step during step setup, the script fails.
- Every `RunParameters.xml` field that has an equivalent step UDF/custom field in Clarity LIMS is required—except for InstrumentName. If a field is not present in the `RunParameters.xml` file, Clarity LIMS logs an error to this effect.

Master Step Fields

The fields defined on the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) step are listed in the Clarity LIMS Configuration area (Custom Fields > Master Step Fields tab).

The following table shows how the parsed `RunParameters.xml` fields map to step fields configured on the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) step in Clarity LIMS.

Table 254 Fields Parsed by parse_novaseq_run_parameters script

RunParameters.xml Field	Field Name
FlowCellSerialBarcode	Flow Cell ID
FlowCellPartNumber	Flow Cell Part Number
FlowCellLotNumber	Flow Cell Lot Number
FlowCellExpirationdate	Flow Cell Expiration Date
FlowCellMode	Flow Cell Mode
RunId	Run ID
ClusterSerialBarcode	PE Serial Barcode
ClusterPartNumber	PE Part Number
ClusterLotNumber	PE Lot Number
ClusterExpirationdate	PE Expiration Date
ClusterCycleKit	PE Cycle Kit
SbsSerialBarcode	SBS Serial Barcode

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RunParameters.xml Field	Field Name
SbsPartNumber	SBS Part Number
SbsLotNumber	SBS Lot Number
SbsExpirationdate	SBS Expiration Date
SbsCycleKit	SBS Cycle Kit
BufferSerialBarcode	Buffer Serial Barcode
BufferPartNumber	Buffer Part Number
BufferLotNumber	Buffer Lot Number
BufferExpirationdate	Buffer Expiration
OutputRunFolder	Output Folder
WorkflowType	Loading Workflow Type

Read InterOp Metrics Automation

Automatically triggered when the sequencing service detects the `CopyComplete.txt` file has been created, this automation parses the InterOp data generated by the run.

- Read summary metrics are recorded on the library pool. These values are aggregated across all lanes. Some values—for example Yield PF (Gb)—are summed, while others are averaged.
- Per lane metrics are recorded on the measurement outputs.

The `read_interop_metrics` script loads data from the InterOp files and records summary metrics into derived sample UDFs/custom fields in Clarity LIMS. If any field is not configured or cannot be written to, the script fails.

Global Fields

The global fields that are configured to support the parsing of key metrics back into Clarity LIMS are as follows.

- Yield PF (Gb) R1
- Yield PF (Gb) R2
- Reads PF (M) R1
- Reads PF (M) R2
- Cluster Density (K/mm^2) R1
- Cluster Density (K/mm^2) R2
- %PF R1
- %PF R2

- % Bases >=Q30 R1
- % Bases >=Q30 R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- % Aligned R1
- % Aligned R
- % Error Rate R1
- % Error Rate R2

Set Next Steps Automation

Automatically triggered on exit of the Record Details screen, the `nextStep = ::ADVANCE::` automation sets the next step to ADVANCE and the samples complete the protocol.

Update Lane Number Automation

Automatically triggered on exit of the Step Setup screen, this automation updates the lane number so that Read InterOp Metrics automation script can populate lane metric to the correct lane.

> Do not disable Update Lane Number automation as it causes run metric parsing to work improperly.

Components Installed

The following sections describe the various components that are installed by default as part of this integration. These components include files, database properties, reagent categories/label groups, reagent kits, and containers. Information on installed workflows, protocols, steps, and automation points is provided in the previous *Workflows, Protocols, and Steps* section.

The Illumina NovaSeq 6000 Integration v2.4.1 Package is distributed as two RPM packages:

- BaseSpaceLIMS-novaseq-extensions
- BaseSpaceLIMS-novaseq-sequencing-service

The RPM packages install the components listed in the following table.

The NovaSeq 6000 Integration requires installation of NGS Extensions Package v5.21 or later.

- If the NGS Extensions Package is not already installed, or if a version earlier than v5.21 is installed, the latest version is installed by default with the NovaSeq integration. See Release Notes for details.

- If NGS Extensions Package v5.21 is already installed, upgrade is not forced or required.
- Secret Util Package is required for the NGS Extensions Package v5.21.
- If the Secret Util Package is not already installed, then Secret Util package is installed along with the installation of NGS Extension Package v5.21.
- If the Secret Util Package is already installed, upgrade is not forced or required.

Table 255 Illumina NovaSeq 6000 Integration v2.4.1 RPM Components

Files Installed	Location and Description
`configure_extensions_novaseq.sh`	`/opt/gls/clarity/config/` Script that imports Clarity LIMS configuration into the application database and updates property values.
`novaseq-extensions.jar`	`/opt/gls/clarity/extensions/novaseq` Jar file containing API-based Clarity LIMS extensions used throughout the protocols.
`novaseq-sequencing.jar`	`/opt/gls/clarity/extensions/novaseq/SequencingService` Sequencing service jar file that captures the run results.
`novaseq-remote-extensions.jar`	`/opt/gls/clarity/extensions/novaseq` Jar file containing scripts that require access to the run folders on the NAS.
InterOp libraries	`/opt/gls/clarity/extensions/novaseq/lib` Illumina shared library for parsing InterOp data files.

`NovaSeq_Standard_Bulk_Pool1.csv`
`NovaSeq_Standard_Bulk_Pool2.csv`
`NovaSeq_Standard_Bulk_Pool3.csv`
`NovaSeq_Xp_Bulk_Pool.csv`
`NovaSeq_Xp_Bulk_Pool2.csv`
`NovaSeq_Xp_Working_Pool.csv`
`NovaSeq_Xp_Working_Pool2.csv`
	`/opt/gls/clarity/extensions/novaseq/Templates`
	Template files used for file generation throughout the protocols.

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Database Properties

The following table lists the database properties installed with the Illumina NovaSeq 6000 Integration v2.4.1 Package.

Table 256 Database Properties Installed

Property	Description
novaseq.runSetupFolder	The directory where the run recipe files are written.
novaseq.sampleSheetPathPrefixSearch.SUFFIX	The directory where the instrument control software or analysis software looks for sample sheets, typically a Windows path to a network drive.
novaseq.sampleSheetPathPrefixReplace.SUFFIX	The directory where the sample sheets are written.
novaseq.sampleSheetPathPrefixSearchReplaceSuffixes*	Search and replace entries for transforming Windows to Linux network paths. This property must be set to the single SUFFIX used in the two previous properties.
novaseq.seqservice.netPathPrefixSearch.SUFFIX	The search prefix used to map the OutputRunFolder field in `RunParameters.xml` to the corresponding Linux directory.
novaseq.seqservice.netPathPrefixReplace.SUFFIX	The directory where the sequencing service looks for run folders.
novaseq.seqservice.netPathPrefixSearchReplaceSuffixes[1]	Search and replace entries for transforming Windows to Linux network paths. This property must be set to the single SUFFIX used in the two properties above.

[1]This version of the integration only supports a single active search-replace pair of each type:sampleSheetPathPrefixSearchReplaceSuffixes and netPathPrefixSearchReplaceSuffixesmust each contain a single number, rather than a comma-separated list of numbers.

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Label Groups, Reagent Kits, and Containers

The NovaSeq 6000 Integration v2.4.1 Package installs the following label groups, reagent kits, and container types.

Label Groups

- TruSeq HT Adapters v2 (D7-D5)

Reagent Kits

- DPX1
- DPX2
- DPX3
- Resuspension Buffer (RSB)
- NaOH
- Tris HCl

Container Types

- Library Tube

Instrument Integration

The following are instructions for configuring the NovaSeq instrument for the Clarity LIMS integration:

1. Launch NovaSeq Control Software.
2. On the home screen, select the menu (top left) and select Settings.
3. Select File-Based and enter the network folder location that NovaSeq Control Software retrieves the JSON recipe from.
4. Enter the network output folder location.
5. Select Save.

For more information, refer to the *NovaSeq Sequencing System Guide*.

Routing Script Requirements

The requirements for the routing script functionality are as follows.

- On the steps that use the routing script—Define Run Format (NovaSeq 6000 v2.3) and Dilute and Denature (NovaSeq 6000 v2.3)—the Next Step for all samples must be set to Remove from workflow. This value is set by a script. Do not change this value in the Assign Next Steps screen.
- In the protocol configuration screen, the following setting is required and must not be changed.
  - In the Next Steps section for the last step in the protocol, the method of assigning the next step must be set to Automatic.

Sample Sheet and Run Recipe File Generation

Sample sheet and run recipe file generation occurs on the step before the run—Dilute and Denature (NovaSeq 6000 v2.3) in the NovaSeq Standard protocol or Load to Flowcell (NovaSeq 6000 v2.3) in the NovaSeq Xp protocol. This step is where samples are placed on the library tube or flow cell that is loaded in the NovaSeq instrument.

In the default configuration, the Generate Sample Sheet and Run Recipe automation generates the following:

- One CSV format sample sheet file for use bcl2fastq v2.20 downstream analysis
- One JSON format run recipe file

The Prepare Files for NovaSeq automation places the generated files on the NAS where the instrument uses them to start the run.

How It Works

- The Prepare Files for NovaSeq automation command line invokes the `copy_attachments_to_network_folder` script `in novaseq-remote-extensions.jar`. The script can be used on a remote AI node/automation worker, in case the Clarity LIMS server does not have direct access to the NAS.
- The output folder for the run recipe file is found in the `novaseq.runSetupFolder` property.
- The output folder for the sample sheet is determined by using the `novaseq.sampleSheetPathSearchReplaceSuffixes` property. This property must contain exactly one suffix (not a comma-separated list) to derive a full property name based on `novaseq.sampleSheetPathPrefixReplace`.

Sample Sheet Contents

The contents of the sample sheet are controlled by the following fields configured on the Dilute and Denature (NovaSeq 6000 v2.3) and Load to Flowcell (NovaSeq 6000 v2.3) steps:

- Experiment Name
- Read 1 Cycles
- Read 2 Cycles
- Workflow
- Reverse Complement Workflow
- Read1UMILength
- Read2UMILength
- Read1StartFromCycle
- Read2StartFromCycle

Refer to the *Bcl2fastq2 Sample Sheet Generation* section of the *Illumina Instrument Sample Sheets (NGS v5.17 & later)* on page 2542 document for detailed information on the following:

- Sample sheet generation script parameters and usage
- Sample sheet data and configuration options
- Enabling unique FASTQ file names per sequencing run

Run Recipe Contents

The contents of the run recipe are controlled by the following fields. These fields are configured on the Dilute and Denature (NovaSeq 6000 v2.3) and Load to Flowcell (NovaSeq 6000 v2.3) steps.

- Experiment Name
- Run Mode
- Workflow Type
- Sample Loading Type (populated based on Workflow field)
- Paired End
- Read 1
- Read 2
- Index Read 1
- Index Read 2
- Sample Sheet Path
- BaseSpace Configuration
- Use Custom Read 1 Primer
- Use Custom Read 2 Primer
- Use Custom Index Read 1 Primer

The following fields are also included in the run recipe:

- Library Tube Barcode (librarytube_ID in JSON file)
  - NovaSeq Standard workflow—Library tube barcode is derived from the container name entered or scanned on the Placement screen.
  - NovaSeq Xp workflow—Library tube barcode is derived from the Library Tube Barcode field in the Load To Flowcell (NovaSeq 6000 v2.3) step.
- Flowcell Barcode (flowcell_ID in JSON file)
  - NovaSeq Standard workflow—Library tube barcode is not output to JSON recipe file.
  - NovaSeq Xp workflow—Flow cell barcode is derived from the container name entered or scanned on the Placement screen.

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- Rehyb—preset to False.
- Output Folder (output_folder in JSON file)—Derived from the system property novaseq.seqservice.netPathPrefixSearch.
- Attachment—Derived from the system property novaseq.sampleSheetPathPrefixSearch with the name of the sample sheet added to the end of the path.
- Use BaseSpace (use_basespace in JSON file)—Set to False if the BaseSpace Sequence Hub Configuration step field value is Not Used, and true otherwise.

Rules and Constraints

- The sample sheet and run recipe files are mandatory inputs for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) step. For the automated run to start successfully, select the Generate Sample Sheet and Run Recipe button to generate these files.
- The output folders must exist on the system where the script runs (can be a remote AI node/automation worker).
- The output folders must be writable by the glsai user.

Container Types

The integration supports the following container types:

- Library tube with barcode provided in the following format:
  - [A-Z]{2}[0-9]{7}-[A-Z]{3}
  - Example—NV1234567-LIB
- Flow cell with barcode provided in one of the following formats:
  - For SP and S1—[A-Z0-9]{5}DR[A-Z0-9]{2}
  - For S2—[A-Z0-9]{5}DM[A-Z0-9]{2}
  - For S4—[A-Z0-9]{5}DS[A-Z0-9]{2}
  - Example S2 flow cell barcode—H1234DMXX

Rules and Constraints

- The workflow configuration contains several validation checks. To make sure that the calculations work properly, it is important that you do not disable any of this validation logic. The validation checks the following information:
  - Which samples, and how many, can enter each step together.
  - Which samples, and how many, can be pooled together.
- Reagent labels (indexes) must be unique.
- The library tube ID must be unique. There should not be multiple library tube containers in the system with the same name.

- Only controls are permitted as unindexed samples. All other unindexed samples and pools are not permitted.
- For sample sheet generation constraints, refer to *Bcl2fastq2 Sample Sheet Generation (NGS v5.16 and earlier)*.
- Do not manually start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) step. This step is fully automated. If the samples have been manually started, the sequencing service does not update them correctly.
- The output folder must be readable by the Clarity LIMS glsjboss account on the server.

NovaSeq 6000 Integration v2.4.1 Validation and Troubleshooting

This guide explains how to validate the installation of the Illumina NovaSeq 6000 Integration Package v2.4.1. The validation process involves the following actions:

- Running samples through the NovaSeq Validation Library Prep (NovaSeq 6000 v2.3) workflow. The workflow contains a single-step protocol that models the library prep required to produce normalized libraries. At the end of the step, the normalized libraries are automatically advanced to the NovaSeq 6000 v2.3 workflow.
- Running normalized libraries through the NovaSeq 6000 v2.3 workflow. This process validates the following details:
  - Successful routing of samples from the Run Format (NovaSeq 6000 v2.3) step to the NovaSeq Standard (NovaSeq 6000 v2.3) or NovaSeq Xp (NovaSeq 6000 v2.3) step.
  - Automated generation of a sample sheet for use with bcl2fastq2 v2.20 analysis software.
  - Automated generation of a run recipe file (JSON file) with the library tube or flow cell barcode as the name (eg, NV1234567-LIB.json or H1234DRXX.json). This file is automatically uploaded to the sequencing instrument and used to set up and initiate the run.
  - Automated tracking of the NovaSeq sequencing run and parsing of run statistics (per run per lane) into the LIMS.

Before executing the validation steps, make sure that you have installed the Illumina NovaSeq 6000 Integration Package v2.4.1 and have imported the default Clarity LIMS configuration.

Activate Workflow, Create Project, Add and Assign Samples

The following steps set up Clarity LIMS in preparation for running samples through the NovaSeq Validation Library Prep (NovaSeq 6000 v2.3) and NovaSeq 6000 v2.3 workflows.

1. In the Clarity LIMS Cconfiguration area, activate the NovaSeq Validation Library Prep (NovaSeq 6000 v2.3) and NovaSeq 6000 v2.3 workflows.
2. On the Projects and Samples screen, create a project and add samples to it.

3. Assign the samples to the NovaSeq Validation Library Prep (NovaSeq 6000 v2.3) workflow.

Library Prep Protocol: NovaSeq Validation Library Prep (NovaSeq 6000 v2.3)

This single-step protocol models the library prep required to produce normalized libraries that are ready for the NovaSeq 6000 v2.3 workflow.

Run NovaSeq Validation Library Prep (NovaSeq 6000 v2.3)

1. In Lab View, locate the NovaSeq Validation Library Prep (NovaSeq 6000 v2.3) protocol. The samples are queued for the NovaSeq Validation Library Prep (NovaSeq 6000 v2.3) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Placement screen, scan or enter the barcode of the destination container into the Container field of the Placed Samples area.
   a. Select the samples from the Samples to be Placed area on the left, then drag them over to the container wells on the right.
   b. Select Add Labels.
5. On the Add Labels screen, select labels from the Labels to Add list and drag them to the container on the right to assign them to placed samples.
   The TruSeq HT Adapters v2 (D7-D5) label group is the only label group configured in the out-of-the-box integration and is the default in this step.
   a. Select Record Details.
6. On the Record Details screen in the Sample Details table, enter values for Normalized Molarity. Type values individually or use the copy/paste functionality as follows.
   a. Select the Copy button to copy the sample information from Clarity LIMS to the clipboard.
   b. Open a blank Excel document and paste the contents of the clipboard into the spreadsheet.
   c. In the spreadsheet, enter the Normalized Molarity values. Select the entire spreadsheet, including the headers (Ctrl + A), and copy (Ctrl + C) the information to the clipboard.
   d. In Clarity LIMS, select the Paste button. Paste the contents of the clipboard (Ctrl + V) into the Update Table window, then select Update to paste into the Sample Details table.
   The Sequencer Type field indicates the instrument to be used for the sequencing run. In this integration, the value of this field is set to NovaSeq automatically.
7. Select Next Steps.
   This selection triggers the Next Steps automation, which sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step successfully.
8. On the Assign Next Steps screen in the Samples table, the Next Step for all samples is prepopulated with Remove from workflow.

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Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.

9. Select Finish Step.
   On exit from the step, the Routing Script automation is triggered. This automation assigns samples to the first step of the NovaSeq 6000 v2.3 workflow—Define Run Format (NovaSeq 6000 v2.3). This is the only step in Protocol 1: Run Format (NovaSeq 6000 v2.3).

Protocol 1: Run Format (NovaSeq 6000 v2.3)

This protocol includes the Define Run Format (NovaSeq 6000 v2.3) step. The step allows for the assignment of per sample values for Loading Workflow Type, Normalized Molarity, Flowcell Type, and Final Loading Concentration (pM). At the end of the step, samples are routed to the NovaSeq Standard or NovaSeq Xp protocol, according to the selected Loading Workflow Type.

Step 1: Run Define Run Format (NovaSeq 6000 v2.3)

1. In Lab View, locate the Run Format (NovaSeq 6000 v2.3) protocol. The samples are queued for the Define Run Format (NovaSeq 6000 v2.3) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Record Details screen, specify the Sample Details.
   - Loading Workflow Type—Select NovaSeq Standard or NovaSeq Xp from the drop-down list.
   - Normalized Molarity—Enter values or use values copied from the previous step.
5. Populate the following fields:

Values can vary across samples.

   - Flowcell Type—Select SP, S1, S2, or S4.
   - Final Loading Concentration (pM)—Select from the two preset options—225 (for PCR-free workflows) or 400 (for Nano workflows)—or enter a different value.
6. Select Next Steps to trigger the Next Steps automation, which does the following actions:
   - Sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step successfully.
   - Calculates the Minimum Molarity.
   - Checks Normalized Molarity value. For samples with no Normalized Molarity value (eg, an empty value, not including 0), the automation generates an error message stating that the field cannot be empty.
   - Compares the Normalized Molarity value of each sample with the Minimum Molarity value.
7. On the Assign Next Steps screen, review the Sample Details table and make sure that the Next Step for all samples is prepopulated with Remove from workflow.

Next Step must be set to Remove from workflow, regardless of the Loading Workflow Type.

> If the Next Step value is not set to Remove from workflow, the routing script automation cannot advance to the next step.

8. Review the Warning field entries for the samples. If the field indicates that the Normalized Molarity is too low, continue with one of the following options:
   - Return to the Record Details screen and adjust the Normalized Molarity value so that it equals or exceeds the Minimum Molarity value. Set the Loading Workflow Type to NovaSeq Standard or NovaSeq Xp, as applicable.
   - Alternatively, complete the protocol without correcting the Normalized Molarity value. In this case, the samples in question are removed from the Clarity LIMS workflow.
9. Select Finish Step.
   The Routing Script automation is triggered if:
   - Samples with a Loading Workflow Type of Remove from workflow (ie, where Normalized Molarity value is lower than the Minimum Molarity) are removed from the Clarity LIMS workflow.
   - Samples with a Loading Workflow Type of NovaSeq Standard are routed to the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.3) step. This step is the first of two steps in Protocol 2: NovaSeq Standard (NovaSeq 6000 v2.3).
   - Samples with a Loading Workflow Type of NovaSeq Xp are routed to the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.3) step. This step is the first of three steps in Protocol 3: NovaSeq Xp (NovaSeq 6000 v2.3).

Protocol 2: NovaSeq Standard (NovaSeq 6000 v2.3)

In this protocol, samples are pooled and added to the library tube in preparation for the NovaSeq run. The protocol contains two steps:

1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.3)
2. Dilute and Denature (NovaSeq 6000 v2.3)

Step 1: Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.3)

1. In Lab View, locate the NovaSeq Standard (NovaSeq 6000 v2.3) protocol. The samples are queued for the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.3) step.
2. On the Queue screen, add samples of the same flow cell type to the Ice Bucket and select Begin Work.
3. On the Pooling screen, create a pool by dragging samples into the Pool Creator. Only one pool must be created.
   a. Type a name for the pool or accept the default name (Pool #1).
   b. Select Record Details.

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4. On exit from the Pooling screen, the Validate Inputs Flowcell Type and Single Pool is triggered. The automation checks the following information:
   - All samples in the pool have been assigned the same Flowcell Type.
   - Only one pool has been created.
5. On the Record Details screen in the Step Details area, complete the fields as follows.
   a. Enter the value for the Number of Flowcells to Sequence field.
   This value is used in volume calculations to make sure that the volumes are sufficient for the number of times the pool is sequenced.
   b. Enter the value for the Minimum Per Sample Volume (µl) field.
   The value in this field is used to calculate how much of each sample is included in the pool. The field is prepopulated with the configured default value (5 µl), but the value can be edited if necessary.
   c. If necessary, enter the value for the % PhiX (2.5 nM) Spike-In field.
   The value in this field is used to calculate the volume of PhiX v3 control to be included in the pool for the given percentage of spike-in. The field is optional and can be edited.

   Assuming the default Minimum Per Sample Volume (µl) value of 5, for a given batch:
   - If the smallest Per Sample Volume (µl) value is less than 5, the LIMS automatically assigns a value of 5 to the sample's Adjusted Per Sample Volume (µl) field.
   - Clarity LIMS then adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.
6. In the Sample Details table, select the pool icon to view details on the pool composition.
7. Select Calculate Volumes to trigger the Calculate Volumes automation, which performs volume calculations based on the selected Flowcell Type:
   - Calculates Bulk Pool Volume (µl) value.
   - Calculates the Per Sample Volume (µl) value to be added to the pool.
   - Calculates PhiX Volume (µl) value if % PhiX (2.5 nM) Spike-In has value.
   - Calculates the Total Sample Volume (µl) value.
   - If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) value.
   - Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
   - Populates the Volume of Pool to Denature (µl), NaOH Volume (µl) and Tris-HCl Volume (µl) columns of the Sample Details table. (Values are set by a script and are not editable.)
   - The automation also generates the Calculation File (*csv) and attaches it to the step. This file contains volume information about the pool and the individual samples it contains. Select the file to download it, then open it in Excel.

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8. Select Next Steps to trigger the Set Next Step automation.
   This automation sets the next step for samples to ADVANCE, advancing them to the Dilute and Denature (NovaSeq 6000 v2.3) step in the protocol.
9. Select Finish Step.

At the end of this step, the pool of samples automatically advances to the Dilute and Denature (NovaSeq 6000 v2.3) step.

Step 2: Dilute and Denature (NovaSeq 6000 v2.3)

1. In Lab View, locate the NovaSeq Standard (NovaSeq 6000 v2.3) protocol. The pool of samples is queued for the Dilute and Denature (NovaSeq 6000 v2.3) step.
2. Add the samples to the Ice Bucket and select Begin Work.
   At the beginning of the step, the Validate Single Input automation is triggered. This automation checks that there is only one container input to the step.
3. On the Placement screen, drag the pool into the library tube in the Placed Samples area.
   a. Scan or type the barcode of the library tube into the Library Tube field.
   b. Select Record Details.
   On exit of the Placement screen, the Validate Library Tube Barcode automation checks that the library tube barcode conforms to the barcode mask [A-Z]{2}[0-9]{7}-[A-Z]{3}. If not, an error message displays.
4. On the Record Details screen, the Reagent Lot Tracking section tracks the NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. To add and activate reagent lots, complete the following steps:
   a. In a new browser tab, open an additional instance of Clarity LIMS.
   b. Navigate to the Consumables configuration screen.
   c. Select the NaOH reagent kit and select New Lot.
   d. Enter the lot details.
   e. Set the Status of Reagent Lot toggle switch to Active.
   f. Select Save.
   g. Repeat this process to add and activate the Resuspension Buffer and Tris-HCl reagent lots.
   h. Close this browser tab and return to your original working tab. Refresh the page.
5. In the Reagent Lot Tracking section, select from the active lots displayed in each drop-down list. The fields displayed in the Step Details section are used to generate the sample sheet and run recipe files. Some of these fields are autopopulated and some must be completed manually. Refer to the following table for details.

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Table 257 Fields Displayed on Record Details Screen of Denature and Dilute (NovaSeq 6000 v2.3) Step

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. No spaces.
BaseSpace Sequence Hub Configuration	Presets • Not Used • Run Monitoring Only • Run Monitoring and Storage
Workflow	Presets • GenerateFASTQ
Workflow Type	Presets • No Index • Single Index • Dual Index
Index Read 1	Presets • 0 • 6 • 8 • type a value between 0 and 20
Index Read 2	Presets • 0 • 6 • 8 • type a value between 0 and 20
Paired End	Presets • True • False
Read 1 Cycles	Presets • 251 • 151 • 101 • 51 • type a value between 1 and 251 for the SP flow cell type or between 1 and 151 for all other types

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Field	Value
Read 2 Cycles	Presets • 251 • 151 • 101 • 51 • type a value between 0 and 251 for the SP flow cell type or between 0 and 151 for all other types
Use Custom Read 1 Primer	Select if applicable.
Use Custom Read 2 Primer	Select if applicable.
Use Custom Index Read 1 Primer	Select if applicable.
Reverse Complement Workflow	Select Yes (by default) for v1.5 reagents in NovaSeq Control Software v1.7 and onwards. Select No for v1 reagents.
UMI - Read 1 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 1. Leave blank if not applicable.
UMI - Read 2 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 2. Leave blank if not applicable.
UMI - Read 1 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.
UMI - Read 2 Start From Cycle	Enter the cycle number that Read 2 starts from. Leave blank if not applicable.
Sample Sheet Path	Automatically populated after the sample sheet has been generated.

6. On the Record Details screen, select Generate Sample Sheet and Run Recipe. This selection triggers the automation script: The sample sheet and JSON files are generated and attached to the placeholders in the Files area of the Record Details screen.
   The Sample Sheet Path field is populated with the path to the sample sheet file.
7. Select Next Steps. This selection triggers the Prepare Files for NovaSeq automation, which does the following:
   - Copies the sample sheet and run recipe files to the location specified during installation. The NovaSeq instrument software uses these files to set up the run.
   - Sets the value of the next step to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step successfully.

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8. On the Assign Next Steps screen, the Next Step for samples is prepopulated with Remove from workflow.
   Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.
9. Select Finish Step.
   On exit from the step, the Routing Script automation is triggered and samples are routed to Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3).
   In Lab View, the pool of samples is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) step.

> Do not add samples to the Ice Bucket or start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) step. The integration does this action automatically.

Protocol 3: NovaSeq Xp (NovaSeq 6000 v2.3)

In this protocol, samples are pooled and added to lanes on the NovaSeq flow cell. The flow cell type is determined by the option selected in the Define Run Format (NovaSeq 6000 v2.3) step.

The protocol contains three steps:

1. Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.3)
2. Dilute, Denature and ExAmp (NovaSeq 6000 v2.3)
3. Load to Flowcell (NovaSeq 6000 v2.3)

Step 1: Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.3)

1. In Lab View, locate the NovaSeq Xp (NovaSeq 6000 v2.3) protocol. The samples are queued for the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.3) step.
2. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucket and select Begin Work.
3. On the Pooling screen, create a pool by dragging samples into the Pool Creator. Only one pool must be created.
   a. Type a name for the pool or accept the default name Pool #1.
   b. Select Record Details.
   On exit from the Pooling screen, the Validate Inputs Flowcell Type and Single Pool is triggered. The automation checks the following details:
   - All samples in the pool have been assigned the same Flowcell Type.
   - Only one pool has been created.
4. On the Record Details screen in the Step Details area, complete the fields as follows.
   a. Enter the value for the Number of Lanes to Sequence field.
   This value is used in volume calculations to make sure that the volumes are sufficient for the number of times the pool is sequenced.

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b. Enter the value for the Minimum Per Sample Volume (µl) field.
The value in this field is used to calculate how much of each sample is included in the pool. The field is prepopulated with the configured default value (5 µl), but the value can be edited if necessary.

c. If necessary, enter the value for the % PhiX (0.25 nM) Spike-In field.
The value in this field is used to calculate the volume of PhiX v3 control to be included in the pool for the given percentage of spike-in. The field is optional and can be edited.

Assuming the default Minimum Per Sample Volume (µl) value of 5, for a given batch:

- If the smallest Per Sample Volume (µl) value is less than 5, the LIMS automatically assigns a value of 5 to the sample's Adjusted Per Sample Volume (µl) field.
- Clarity LIMS then adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

5. In the Sample Details table, select the pool icon to view details on the pool composition.
6. Select Calculate Volumes to trigger the Calculate Volumes automation, which performs volume calculations based on the selected Flowcell Type:
   - Calculates Bulk Pool Volume (µl) value.
   - Calculates the Per Sample Volume (µl) value to be added to the pool.
   - Calculates PhiX Volume (µl) value if % PhiX (2.5 nM) Spike-In has value.
   - Calculates the Total Sample Volume (µl) value.
   - If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) value.
   - Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
   - The automation also generates the Calculation File (*csv) and attaches it to the step. This file contains volume information about the samples and RSB buffer to add to the pool. Select the file to download it, then open it in Excel.
7. Select Next Steps to trigger the Set Next Step automation.
   This automation sets the next step for samples to ADVANCE, advancing them to the Dilute, Denature and ExAmp (NovaSeq 6000 v2.3) step in the protocol.
8. Select Finish Step.

At the end of this step, the pool of samples automatically advances to the Dilute, Denature and ExAmp (NovaSeq 6000 v2.3) step.

Step 2: Dilute, Denature and ExAmp (NovaSeq 6000 v2.3)

1. In Lab View, locate the NovaSeq Xp (NovaSeq 6000 v2.3) protocol. The pool of samples is queued for the Dilute, Denature and ExAmp (NovaSeq 6000 v2.3) step. Add the pool to the Ice Bucket.

2. On the Ice Bucket screen, set the number of derivatives to create (they are placed into the flow cell lanes) and select Begin Work.
   The Validate Inputs Flowcell Type automation checks that there is only one container input to the step.
3. On entry to the Record Details screen, the Calculate Volumes automation is triggered.
   This automation sets the following values based on the selected Flowcell Type:
   - NaOH Volume (µl)
   - Tris-HCl Volume (µl)
   - DPX1 Volume (µl)
   - DPX2 Volume (µl)
   - DPX3 Volume (µl)
   - Mastermix per Lane (µl)
   - The automation also generates the Calculation File (*csv) and attaches it to the step. This file contains information about the DPX Mastermix volume and the volume of Mastermix, NaOH, and Tris-HCl to add per working pool.
4. On the Record Details screen, the Reagent Lot Tracking section tracks the DPX1, DPX2, DPX3, NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. To add and activate reagent lots, complete the following steps:
   a. In a new browser tab, open an additional instance of Clarity LIMS.
   b. Navigate to the Consumables configuration screen.
   c. Select one of the reagent kits listed and select New Lot.
   d. Enter the lot details.
   e. Set the Status of Reagent Lot toggle switch to Active.
   f. Select Save.
   g. Repeat this process to add and activate the remaining reagent lots.
   h. Close this browser tab and return to your original working tab. Refresh the page.
5. On the Record Details screen in the Reagent Lot Tracking section, select from the active lots displayed in each drop-down list.
   In the Step Details area, the DPX1, DPX2, and DPX3 reagent volume values are already populated. These values are set by a script and are not editable while running the step.
6. In the Sample Details table, select the pool icon to view details on the working pool composition.

The BP Aliquot, Mastermix per lane, NaOH, and Tris-HCl volume values for each working pool are already populated. These values are set by a script and are not editable while running the step.

The working pool number is appended to the bulk pool name. This feature allows you to identify which working pools are derived from the same bulk pool quickly.

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7. [Optional] Select the Calculation File (CSV) in the Files area to view details on the following volumes to add per working pool:
   - DPX Mastermix
   - Mastermix
   - NaOH
   - Tris-HCl
8. Select Next Steps.
   On the Assign Next Steps screen, the next step is already set to Load to Flowcell (NovaSeq 6000 v2.3).
9. Select Finish Step.

Step 3: Run Load to Flowcell (NovaSeq 6000 v2.3)

1. On the Ice Bucket screen in the Container Options panel, select the appropriate flow cell type from the Destination Container drop-down list and select Begin Work.
2. On the Placement screen, drag the pools from the left of the screen over into the Placed Samples area on the right.
   a. Scan or type the barcode of the flow cell into the Flow Cell field.
   b. Select Record Details.
   On exit of the Placement screen, the Validate Flowcell Barcode automation validates the container barcode.

Flowcell Type	Barcode Mask
SP	[A-Z0-9]{5}DR[A-Z0-9]{2}
S1	[A-Z0-9]{5}DR[A-Z0-9]{2}
S2	[A-Z0-9]{5}DM[A-Z0-9]{2}
S4	[A-Z0-9]{5}DS[A-Z0-9]{2}

3. The fields displayed in the Step Details section are used to generate the sample sheet and run recipe files. Some of these fields are autopopulated and some must be completed manually. See the following table for details.

Table 258 Fields Displayed on Record Details Screen of Load to Flowcell (NovaSeq 6000 v2.3) Step

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. No spaces.

Field	Value
BaseSpace Sequence Hub Configuration	Presets • Not Used • Run Monitoring Only • Run Monitoring and Storage
Workflow	Presets • GenerateFASTQ
Workflow Type	Presets • No Index • Single Index • Dual Index
Index Read 1	Presets • 0 • 6 • 8 • type a value between 0 and 20
Index Read 2	Presets • 0 • 6 • 8 • type a value between 0 and 20
Paired End	Presets • True • False
Read 1 Cycles	Presets • 251 • 151 • 101 • 51 • type a value between 1 and 251 for the SP flow cell type or between 1 and 151 for all other types
Read 2 Cycles	Presets • 251 • 151 • 101 • 51 • type a value between 0 and 251 for the SP flow cell type or between 0 and 151 for all other types

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Field	Value
Use Custom Read 1 Primer	Select if applicable.
Use Custom Read 2 Primer	Select if applicable.
Use Custom Index Read 1 Primer	Select if applicable.
Reverse Complement Workflow	Select Yes (by default) for v1.5 reagents in NovaSeq Control Software v1.7 and onwards. Select No for v1 reagents.
UMI - Read 1 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 1. Leave blank if not applicable.
UMI - Read 2 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 2. Leave blank if not applicable.
UMI - Read 1 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.
UMI - Read 2 Start From Cycle	Enter the cycle number that Read 2 starts from. Leave blank if not applicable.
Sample Sheet Path	Automatically populated after the sample sheet has been generated.
Library Tube Barcode	Scan the library tube barcode.

4. Select Generate Sample Sheet and Run Recipe. This selection triggers the automation script, which:
   - Generates and attaches the sample sheet and JSON files to the placeholders in the Files area of the Record Details screen.
   - Populates the Sample Sheet Path field with the path to the sample sheet file.
5. Select Next Steps.
   This selection triggers the Prepare Files for NovaSeq automation, which copies the sample sheet and run recipe files to the location specified during installation. The NovaSeq instrument software uses these files to set up the run.
6. On the Assign Next Steps screen, make sure the Next Step for samples is prepopulated with Mark protocol as complete.
7. Select Finish Step.

At this point in the workflow, the user interaction ends. The flow cell is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) step.

Proceed to Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3).

Do not add samples to the Ice Bucket or start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) step. The integration does this action automatically.

Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3)

This protocol contains a single fully automated step - AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3).

The integration starts the step automatically and data from the run is parsed back into Clarity LIMS. No user interaction is required. However, you can open and review the various stages of the step in Clarity LIMS.

How the Integration Works

1. When initiating a run on the NovaSeq instrument, the NovaSeq Control Software (NVCS) looks for the sample sheet and run recipe (*.json file) on the shared network drive. The NVCS copies the sample sheet to the run directory.
   The NVCS uses the run recipe to start the sequencing run.
2. When the run starts, the instrument software creates a new run folder on the shared network drive and copies the following files into that folder:
   - `RunParameters.xml`
   - `RunInfo.xml`
   - LIMS/ <ContainerID>.json file (run recipe)
   - The Real-Time Analysis v3 (RTA3) software creates the InterOp folder.
3. The sequencing service detects the presence of the `RunParameters`, `RunInfo`, and JSON files and starts the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) step in Clarity LIMS.
4. As the run progresses, the InterOp data files are filled in.
5. At the end of the run, the NovaSeq creates the `CopyComplete.txt` file in the InterOp folder, which indicates to the sequencing service that the run has completed and the data files are ready.
6. The NovaSeq copies the run data files to the InterOp folder and the sequencing service triggers the Read InterOp Metrics automation. This automation reads the files, records the parsed metrics into Clarity LIMS, and completes the step in Clarity LIMS.

Parse Run Parameters and Generate Link to Run Folder Automation

The Parse Run Parameters and Generate Link to Run Folder automation is automatically triggered on entry to the Record Details screen. This automation parses the `RunParameters.xml` file attached on the Step Setup screen and populates the global/master step fields with extracted values. For details on this automation, including how the parsed fields map to the master step fields in Clarity LIMS, refer to *NovaSeq 6000 Integration v2.4.1 Configuration & User Interaction* on page 1002.

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Read InterOp Metrics Automation

Automatically triggered when the sequencing service detects the `CopyComplete.txt` file has been created, this automation parses the InterOp data generated by the run.

The `read_interop_metrics` script records summary metrics into the following global fields in Clarity LIMS. The script fails if any field is not configured or cannot be written to.

- Yield PF (Gb) R1
- Yield PF (Gb) R2
- Reads PF (M) R1
- Reads PF (M) R2
- Cluster Density (K/mm^2) R1
- Cluster Density (K/mm^2) R2
- %PF R1
- %PF R2
- % Bases >=Q30 R1
- % Bases >=Q30 R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- % Aligned R1
- % Aligned R
- % Error Rate R1
- % Error Rate R2

For details, refer to *NovaSeq 6000 Integration v2.4.1 Configuration & User Interaction* on page 1002.

Set Next Steps Automation

After the run metrics are parsed successfully, the Set Next Steps automation sets the next step for all samples to Mark protocol as complete. For details, see the Configuration and User Interaction documentation.

Troubleshooting

If an automation trigger does not appear to run its corresponding scripts, see the following sections in the Clarity LIMS API documentation:

- *Troubleshooting Automated Informatics*
- *Troubleshooting Automation in the API*

If an error occurs that does not provide direction on how to proceed, complete the following steps:

1. Confirm the version of the installed Illumina NovaSeq Integration Package by running the `rpm -qa | grep -i novaseq` command as follows.
   - For an on-premise deployment, run the command on Clarity LIMS server command line. This command retrieves the version of both RPMs installed.
   - For a cloud hosted deployment, run the command on the local server installation. This command retrieves the version of the remote extensions RPM installed.
2. If the error is related to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.3) step, there are two places to check for log file information:
   - If the step does not start, check the `NovaSeqIntegrator.log` file written to:

```
/opt/gls/clarity/extensions/novaseq/SequencingService/NovaSeqIntegrator.log
```

     This log file is written to this path wherever the remote extensions RPM was installed.
   - If the step starts but does not complete, open the step in Clarity LIMS (it can be found via a search). On the Record Details screen, the log file is attached to a placeholder called Log File. If you are unable to reach the Record Details screen, or if the file does not contain an error, review the `NovaSeqIntegrator.log` described previously.
3. Contact Illumina Support and supply the relevant information from the troubleshooting steps already performed.

NovaSeq 6000 Integration v2.4.1 Manual Upgrade

NovaSeq 6000 Integration Package v2.4.1 includes the preconfigured NovaSeq 6000 v2.3 workflow. The integration includes updated formulas used in the calculation scripts and updated flow cell and library tube barcode validation patterns. Clarity LIMS v4.x is no longer supported in NovaSeq 6000 Integration Package v2.4 and later versions.

To keep the v2.2 workflow and upgrade to the latest v2 integration features, you can manually upgrade the configuration.

Before you manually upgrade, make sure the following items are installed:

- NGS Extension Package (v5.21 or later)
- Secret Utility v1.2

The NGS Extension Package v5.21 update is included in the NovaSeq 6000 Integration v2.4.1 upgrade. Secret Utility is also installed as part of NGS Extension Package v5.21.

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Installation Steps

1. Install NGS v5.21 with Secret Utility v1.2.
2. Configure Secret Utility. Refer to the Secret Utility Configuration section that follows.
3. Install the BaseSpaceLIMS-novaseq-extensions v2.4.1 RPM.

   When prompted, do not run the following script:
   `/opt/gls/clarity/config/configure_extensions_novaseq.sh`

4. Install the BaseSpaceLIMS-novaseq-sequencing-service v2.4.1 RPM.
5. Manually upgrade your configuration using the instructions provided in the following Update NovaSeq Standard (NovaSeq 6000 v2.2) Protocol and Update NovaSeq Xp (NovaSeq 6000 v2.2) Protocol sections.

Configure Secret Utility

If Clarity LIMS Secret Utility is not installed or configured, use the following information to configure Secret Utility.

- Installation on a hosted system with Clarity LIMS (v5.4 or later)—The Clarity LIMS installation tooling configures this installation. No additional configuration is necessary.
- Installation on Clarity LIMS v5.3 (or other versions that are v5.1 and later)—The installation includes the following passwords:
  - Tenant DB password
  - Clarity DB password
  - FTP password
  - LDAP manager password (if LDAP is enabled)
  - LabLink DB password (for Clarity LIMS v5.3 LabLink only)
  - Reporting DB password
  - RabbitMQ password

For details on the Secret Utility configuration, refer to *Guide to Secret Management* in the Clarity LIMS Core Functionality documentation.

Update NovaSeq Standard (NovaSeq 6000 v2.2) Protocol

The manual integration upgrade starts with updates to the NovaSeq Standard (NovaSeq 6000 v2.2) Protocol.

1. Update the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.2) step as follows.
   a. In Clarity LIMS, under Configuration, select the Custom Fields tab.
   b. Under the Master Step Fields tab, select the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.2) master step.

c. Select the Calculate Volumes Script custom field.

d. In the Field Details section, update the preset value with the following script:

```
step.::PhiX Volume (ul):: = !step.hasValue(::% PhiX (2.5nM) Spike-In::) ? 0 :
step.::% PhiX (2.5nM) Spike-In::; if (input.::Flowcell Type:: == ::SP::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 100;
step.::PhiX Volume (ul):: *= 0.6 }; if (input.::Flowcell Type:: == ::S1::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 100;
step.::PhiX Volume (ul):: *= 0.6 }; if (input.::Flowcell Type:: == ::S2::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 150;
step.::PhiX Volume (ul):: *= 0.9 }; if (input.::Flowcell Type:: == ::S4::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 310;
step.::PhiX Volume (ul):: *= 1.9 }; if (step.::PhiX Volume (ul):: == 0) {
step.::PhiX Volume (ul):: = :::: }; input.::Per Sample Volume (ul):: =
(((input.::Final Loading Concentration (pM):: * 5 / 1000) / input.::Normalized
Molarity (nM)::) * step.::Bulk Pool Volume (ul)::) / step.::Number of Samples in
Pool::
```

e. Select Save.

2. Modify the Calculate Volumes automation on the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.2) step as follows.

a. From Configuration, select the Automation tab.

b. Under the Step Automation tab, select the Calculate Volumes automation that is enabled on the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.2) master step.

c. Update the default command line to the value provided in the drop-down section that follows.

Update Calculate Volumes Command Line

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
-t true -h false -log {compoundOutputFileLuid1} \
script:evaluateDynamicExpression \
-exp 'step.::Number of Samples in Pool:: = step.::Number of Samples in Pool::
+ 1' \
script:evaluateDynamicExpression \
-exp '{udf:Calculate Volumes Script}' \
&& /opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/novaseq/novaseq-extensions.jar script:calculate_
adjusted_per_sample_volume -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
-t true -h false -log {compoundOutputFileLuid1} \
```


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```
script:evaluateDynamicExpression \
-exp 'step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: +
input.::Adjusted Per Sample Volume (ul)::' \
script:evaluateDynamicExpression \
-exp 'if (step.::Total Sample Volume (ul):: >= step.::Bulk Pool Volume
(ul)::) {output.::RSB Volume (ul):: = 0} else {output.::RSB Volume (ul):: =
step.::Bulk Pool Volume (ul):: - step.::Total Sample Volume (ul)::} ;
output.::Flowcell Type:: = input.::Flowcell Type:: ; output.::Loading
Workflow Type:: = input.::Loading Workflow Type:: ; if ( input.::Flowcell
Type:: == ::SP:: ) { output.::Volume of Pool to Denature (ul):: = 100 ;
output.::NaOH Volume (ul):: = 25 ; output.::Tris-HCl Volume (ul):: = 25 } ;
if ( input.::Flowcell Type:: == ::S1:: ) { output.::Volume of Pool to
Denature (ul):: = 100 ; output.::NaOH Volume (ul):: = 25 ; output.::Tris-HCl
Volume (ul):: = 25 } ; if ( input.::Flowcell Type:: == ::S2:: )
{output.::Volume of Pool to Denature (ul):: = 150 ; output.::NaOH Volume
(ul):: = 37 ; output.::Tris-HCl Volume (ul):: = 38 } ; ; if (
input.::Flowcell Type:: == ::S4:: ) { output.::Volume of Pool to Denature
(ul):: = 310 ; output.::NaOH Volume (ul):: = 77 ; output.::Tris-HCl Volume
(ul):: = 78 }' \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {processURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Standard_Bulk_
Pool1.csv \
-o 1.csv \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Standard_Bulk_
Pool2.csv \
-o 2.csv \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Standard_Bulk_
Pool3.csv \
-o 3.csv \
&& cat 1.csv 2.csv 3.csv > {compoundOutputFileLuid0}.csv \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
-t true -log {compoundOutputFileLuid1} \
script:evaluateDynamicExpression \
-h false \
```


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```
-exp 'step.::Number of Samples in Pool:: = step.::Number of Samples in Pool::
- 1; step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: -
input.::Adjusted Per Sample Volume (ul)::'"
```

You can also update the existing automation by replacing the fourth -exp section in the command line with the following code. The changes are highlighted in red.

```
if (step.::Total Sample Volume (ul):: >= step.::Bulk Pool Volume (ul)::)
{output.::RSB Volume (ul):: = 0} else {output.::RSB Volume (ul):: =
step.::Bulk Pool Volume (ul):: - step.::Total Sample Volume (ul)::} ;
output.::Flowcell Type:: = input.::Flowcell Type:: ; output.::Loading
Workflow Type:: = input.::Loading Workflow Type:: ; if ( input.::Flowcell
Type:: == ::SP:: ) { output.::Volume of Pool to Denature (ul):: = 100 ;
output.::NaOH Volume (ul):: = 25 ; output.::Tris-HCl Volume (ul):: = 25 } ;
if ( input.::Flowcell Type:: == ::S1:: ) { output.::Volume of Pool to
Denature (ul):: = 100 ; output.::NaOH Volume (ul):: = 25 ; output.::Tris-HCl
Volume (ul):: = 25 } ; if ( input.::Flowcell Type:: == ::S2:: )
{output.::Volume of Pool to Denature (ul):: = 150 ; output.::NaOH Volume
(ul):: = 37 ; output.::Tris-HCl Volume (ul):: = 38 } ; ; if (
input.::Flowcell Type:: == ::S4:: ) { output.::Volume of Pool to Denature
(ul):: = 310 ; output.::NaOH Volume (ul):: = 77 ; output.::Tris-HCl Volume
(ul):: = 78 }
```

d. Select Save.

e. Make sure the start of the script contains `-exp '{udf:Calculate Volumes Script}' \` and that the bottom of the script matches the content of the Updated Calculate Volumes Script drop-down section.

3. Update the Validate Library Tube Barcode automation on the Dilute and Denature (NovaSeq 6000 v2.2) step as follows.

a. From Configuration, select the Automation tab.

b. Under the Step Automation tab, select the Validate Library Tube Barcode automation that is enabled on the Dilute and Denature (NovaSeq 6000 v2.2) master step.

c. Update the command line with the following script:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
script:evaluateDynamicExpression -exp 'if (!output.container.name.matches( ::[A-
Z]{2}[0-9]{7}-[A-Z]{3}:: ) ) {fail ( ::Invalid Library Tube Barcode. Please
verify and try again.:: ) }' -log {compoundOutputFileLuid2}"
```

d. Select Save.

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Update NovaSeq Xp (NovaSeq 6000 v2.2) Protocol

The manual integration upgrade continues with updates to the NovaSeq Xp (NovaSeq 6000 v2.2) protocol.

1. Update the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.2) step as follows.
   a. From Configuration, select the Automation tab.
   b. Under the Step Automation tab, select the Calculate Volumes automation that is enabled on the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.2) master step.
   c. Update the command line to the default value provided in the drop-down section that follows.

Update Calculate Volumes Command Line

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
-t true -h false -log {compoundOutputFileLuid1} \
script:evaluateDynamicExpression \
-exp 'step.::Number of Samples in Pool:: = step.::Number of Samples in Pool::
+ 1' \
script:evaluateDynamicExpression \
-exp 'step.::PhiX Volume (ul):: = !step.hasValue(::% PhiX (0.25nM) Spike-
In::) ? 0 : step.::% PhiX (0.25nM) Spike-In::; if (input.::Flowcell Type:: ==
::SP::) { step.::Bulk Pool Volume (ul):: = step.::Number of Lanes to
Sequence:: * 18; step.::PhiX Volume (ul):: *= 0.7 }; if (input.::Flowcell
Type:: == ::S1::) { step.::Bulk Pool Volume (ul):: = step.::Number of Lanes
to Sequence:: * 18; step.::PhiX Volume (ul):: *= 0.7 }; if (input.::Flowcell
Type:: == ::S2::) { step.::Bulk Pool Volume (ul):: = step.::Number of Lanes
to Sequence:: * 22; step.::PhiX Volume (ul):: *= 0.8 }; if (input.::Flowcell
Type:: == ::S4::) { step.::Bulk Pool Volume (ul):: = step.::Number of Lanes
to Sequence:: * 30; step.::PhiX Volume (ul):: *= 1.1 }; if (step.::PhiX
Volume (ul):: == 0) { step.::PhiX Volume (ul):: = :::: }; input.::Per Sample
Volume (ul):: = (((input.::Final Loading Concentration (pM):: * 5 / 1000) /
input.::Normalized Molarity (nM)::) * step.::Bulk Pool Volume (ul)::) /
step.::Number of Samples in Pool::' \
&& /opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/novaseq/novaseq-extensions.jar script:calculate_
adjusted_per_sample_volume -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
-t true -h false -log {compoundOutputFileLuid1} \
script:evaluateDynamicExpression \
```


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```
-exp 'step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: +
input.::Adjusted Per Sample Volume (ul)::' \
script:evaluateDynamicExpression \
-exp 'if (step.::Total Sample Volume (ul):: >= step.::Bulk Pool Volume
(ul)::) {output.::RSB Volume (ul):: = 0} else {output.::RSB Volume (ul):: =
step.::Bulk Pool Volume (ul):: - step.::Total Sample Volume (ul)::} ;
output.::Flowcell Type:: = input.::Flowcell Type:: ; output.::Loading
Workflow Type:: = input.::Loading Workflow Type::' \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {processURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Xp_Bulk_Pool.csv \
-o {compoundOutputFileLuid0}.csv \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Xp_Bulk_Pool2.csv \
-o append.csv \
&& cat append.csv >> {compoundOutputFileLuid0}.csv \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
-t true -log {compoundOutputFileLuid1} \
script:evaluateDynamicExpression \
-h false \
-exp 'step.::Number of Samples in Pool:: = step.::Number of Samples in Pool::
- 1; step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: -
input.::Adjusted Per Sample Volume (ul)::'"
```

You can also update the second -exp line with the following code. Changes are highlighted in red.

```
step.::PhiX Volume (ul):: = !step.hasValue(::% PhiX (0.25nM) Spike-In::) ? 0
: step.::% PhiX (0.25nM) Spike-In::; if (input.::Flowcell Type:: == ::SP::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Lanes to Sequence:: * 18;
step.::PhiX Volume (ul):: *= 0.7 }; if (input.::Flowcell Type:: == ::S1::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Lanes to Sequence:: * 18;
step.::PhiX Volume (ul):: *= 0.7 }; if (input.::Flowcell Type:: == ::S2::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Lanes to Sequence:: * 22;
step.::PhiX Volume (ul):: *= 0.8 }; if (input.::Flowcell Type:: == ::S4::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Lanes to Sequence:: * 30;
step.::PhiX Volume (ul):: *= 1.1 }; if (step.::PhiX Volume (ul):: == 0) {
step.::PhiX Volume (ul):: = :::: }; input.::Per Sample Volume (ul):: =
```

```
(((input.::Final Loading Concentration (pM):: * 5 / 1000) /
input.::Normalized Molarity (nM)::) * step.::Bulk Pool Volume (ul)::) /
step.::Number of Samples in Pool::
```

d. Select Save.

2. Update the Calculate Volumes automation on the Dilute, Denature, & ExAmp (NovaSeq 6000 v2.2) step as follows.
   a. From Configuration, select the Automation tab.
   b. Under the Step Automation tab, select the Calculate Volumes automation that is enabled on the Dilute, Denature, & ExAmp (NovaSeq 6000 v2.2) master step.
   c. Update the command line to the default value provided in the drop-down section that follows.

Update Calculate Volumes Command Line

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
\
script:evaluateDynamicExpression \
-t true \
-h false \
-exp 'if ( input.::Flowcell Type:: == ::SP:: ) { output.::BP Aliquot Volume
(ul):: = 18 ; output.::NaOH Volume (ul):: = 4 ; output.::Tris-HCl Volume
(ul):: = 5 ; step.::DPX1 Volume (ul):: = 126 ; step.::DPX2 Volume (ul):: = 18
; step.::DPX3 Volume (ul):: = 66 ; output.::Mastermix per Lane (ul):: = 63}
;if ( input.::Flowcell Type:: == ::S1:: ) { output.::BP Aliquot Volume (ul)::
= 18 ; output.::NaOH Volume (ul):: = 4 ; output.::Tris-HCl Volume (ul):: = 5
; step.::DPX1 Volume (ul):: = 126 ; step.::DPX2 Volume (ul):: = 18 ;
step.::DPX3 Volume (ul):: = 66 ; output.::Mastermix per Lane (ul):: = 63} ;
if ( input.::Flowcell Type:: == ::S2:: ) { output.::BP Aliquot Volume (ul)::
= 22 ; output.::NaOH Volume (ul):: = 5 ; output.::Tris-HCl Volume (ul):: = 6
; step.::DPX1 Volume (ul):: = 126 ; step.::DPX2 Volume (ul):: = 18 ;
step.::DPX3 Volume (ul):: = 66 ; output.::Mastermix per Lane (ul):: = 77} ;
if ( input.::Flowcell Type:: == ::S4:: ) { output.::BP Aliquot Volume (ul)::
= 30 ; output.::NaOH Volume (ul):: = 7 ; output.::Tris-HCl Volume (ul):: = 8
; step.::DPX1 Volume (ul):: = 315 ; step.::DPX2 Volume (ul):: = 45 ;
step.::DPX3 Volume (ul):: = 165 ; output.::Mastermix per Lane (ul):: = 105} ;
output.::Flowcell Type:: = input.::Flowcell Type:: ; output.::Loading
Workflow Type:: = input.::Loading Workflow Type::' \
-log {compoundOutputFileLuid1} \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {processURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} \
```


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```
script:driver_file_generator \
-t /opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Xp_Working_Pool.csv
\
-o {compoundOutputFileLuid0}.csv \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Xp_Working_Pool2.csv
\
-o append.csv \
&& cat append.csv >> {compoundOutputFileLuid0}.csv"
```

You can also update the existing automation by replacing the first -exp line with the following code. Changes are highlighted in red.

```
if ( input.::Flowcell Type:: == ::SP:: ) { output.::BP Aliquot Volume (ul)::
= 18 ; output.::NaOH Volume (ul):: = 4 ; output.::Tris-HCl Volume (ul):: = 5
; step.::DPX1 Volume (ul):: = 126 ; step.::DPX2 Volume (ul):: = 18 ;
step.::DPX3 Volume (ul):: = 66 ; output.::Mastermix per Lane (ul):: = 63} ;if
( input.::Flowcell Type:: == ::S1:: ) { output.::BP Aliquot Volume (ul):: =
18 ; output.::NaOH Volume (ul):: = 4 ; output.::Tris-HCl Volume (ul):: = 5 ;
step.::DPX1 Volume (ul):: = 126 ; step.::DPX2 Volume (ul):: = 18 ;
step.::DPX3 Volume (ul):: = 66 ; output.::Mastermix per Lane (ul):: = 63} ;
if ( input.::Flowcell Type:: == ::S2:: ) { output.::BP Aliquot Volume (ul)::
= 22 ; output.::NaOH Volume (ul):: = 5 ; output.::Tris-HCl Volume (ul):: = 6
; step.::DPX1 Volume (ul):: = 126 ; step.::DPX2 Volume (ul):: = 18 ;
step.::DPX3 Volume (ul):: = 66 ; output.::Mastermix per Lane (ul):: = 77} ;
if ( input.::Flowcell Type:: == ::S4:: ) { output.::BP Aliquot Volume (ul)::
= 30 ; output.::NaOH Volume (ul):: = 7 ; output.::Tris-HCl Volume (ul):: = 8
; step.::DPX1 Volume (ul):: = 315 ; step.::DPX2 Volume (ul):: = 45 ;
step.::DPX3 Volume (ul):: = 165 ; output.::Mastermix per Lane (ul):: = 105} ;
output.::Flowcell Type:: = input.::Flowcell Type:: ; output.::Loading
Workflow Type:: = input.::Loading Workflow Type::
```

d. Select Save.

3. Update the Validate Flowcell Barcode automation on the Load to Flowcell (NovaSeq 6000 v2.2) step as follows.
   a. From Configuration, select the Automation tab.
   b. Under the Step Automation tab, select the Validate Flowcell Barcode automation that is enabled on the Load to Flowcell (NovaSeq 6000 v2.2) master step.
   c. Update the command line with the following script:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
```


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```
-exp 'if (input.::Flowcell Type:: == ::SP:: && !output.container.name.matches(
::[A-Z0-9]{5}DR[A-Z0-9]{2}:: ) ) {fail ( ::Invalid Flowcell Barcode. Please
verify and try again.:: ) } ; if (input.::Flowcell Type:: == ::S1:: &&
!output.container.name.matches( ::[A-Z0-9]{5}DR[A-Z0-9]{2}:: ) ) {fail (
::Invalid Flowcell Barcode. Please verify and try again.:: ) } ; if
(input.::Flowcell Type:: == ::S2:: && !output.container.name.matches( ::[A-Z0-9]
{5}DM[A-Z0-9]{2}:: ) ) {fail ( ::Invalid Flowcell Barcode. Please verify and try
again.:: ) } ; if (input.::Flowcell Type:: == ::S4:: &&
!output.container.name.matches( ::[A-Z0-9]{5}DS[A-Z0-9]{2}:: ) ) {fail (
::Invalid Flowcell Barcode. Please verify and try again.:: ) } ;
output.::Flowcell Type:: = input.::Flowcell Type::' \
-log {compoundOutputFileLuid2}"
```

d. Select Save.

NovaSeq 6000 Integration v2.4.1 Release Notes

These Release Notes describe the key changes to software components for NovaSeq 6000 Integration Package v2.4.1.

Compatibility

This version of the integration package is compatible with the following software:

- Clarity LIMS v5.1 and later
- NGS Extensions Package v5.21 and later
- NovaSeq Control Software (NVCS) v1.8.0
- Illumina bcl2fastq v2.20

New Features

Features provided with this release of the integration package are as follows:

- Updates to the Validate Flowcell Barcode automation (in the Load to Flowcell step of the NovaSeq Xp protocol) to support future flow cell barcode formats.

Defects Fixed

- Fixed the Validate Flowcell Barcode automation (in the Load to Flowcell Step of NovaSeq Xp Protocol) to accept only the upper case alphanumeric barcode format.
- Fixed the following Calculate Volumes automation script values to sync with the *NovaSeq 6000 System Denature and Dilute Libraries Guide (document # 1000000106351)*:
  - PhiX
  - NaOH
  - Tris-HCl

- Bulk pool volume

Known Issues

None

NovaSeq 6000 Integration v2.4 Configuration & User Interaction

The Illumina NovaSeq 6000 Integration Package v2.4 supports the integration of Clarity LIMS to Illumina NovaSeq 6000 instruments. Clarity LIMS v4.x is no longer supported in NovaSeq 6000 Integration Package v2.4 or in future packages.

This document describes the integration and includes information about the preconfigured protocols, steps, automations, installed components, configuration requirements, rules, and constraints.

For instructions on validating and troubleshooting the Illumina NovaSeq 6000 Integration, see *NovaSeq 6000 Integration v2.4 Validation and Troubleshooting* on page 1140.

The configuration provided in this integration has been established to support NovaSeq 6000 lab processes. Any configuration changes to protocols or workflows—including renaming protocols, steps, and fields—could break the process.

Integration Functionality

The integration includes the following functionality:

- Preconfigured NovaSeq 6000 v2.2 workflow that maps to lab protocols and instrument runs.
- Preconfigured NovaSeq Standard (NovaSeq 6000 v2.2) protocol that supports the loading of pooled libraries into a library tube.
- Preconfigured NovaSeq Xp (NovaSeq 6000 v2.2) protocol that supports individual lane loading on the NovaSeq.
- Automated generation of a sample sheet for use with bcl2fastq2 v2.20 analysis software.
- Automatic generation of run recipe file (JSON format) that is automatically uploaded to the sequencing instrument and used to set up and initiate the run.
- Automated tracking of the NovaSeq sequencing run and parsing of run statistics (per run per lane) into Clarity LIMS, including:
  - Progress and metrics of sequencing run
  - Per instrument sequencing runs (tracked as part of the Run ID field value)
  - Sequencing run parameters
  - Real-Time Analysis v3 (RTA3) run directory location and other run-specific information

- Preconfigured NovaSeq Validation Library Prep (NovaSeq 6000 v2.2) workflow used for validation purposes only. The workflow contains a single-step protocol that models the library prep required to produce normalized libraries that are ready for the NovaSeq 6000 v2.2 workflow. For details, see *NovaSeq 6000 Integration Package v2.4 Validation and Troubleshooting*.
- Preconfigured NovaSeq 6000 v2.2 supports NVCS v1.7 with the following new functions:
  - Unique Molecular Identifier (UMI) settings
  - Reverse complement workflow and reverse complement sample sheet generation
  - Supports new NovaSeq reagent kit v1.5
- Preconfigured NovaSeq 6000 v2.2 adds support for NovaSeq SP flow cell.

Information on these features is provided in the following sections.

Prerequisites and Assumptions

It is assumed that samples enter the NovaSeq 6000 v2.2 workflow as normalized libraries. That is, before they are assigned to the workflow the following actions occur:

- Samples have been accessioned into Clarity LIMS.
- Samples have been run through QC and library prep.
- Samples have been normalized, and the value is captured in a field called Normalized Molarity (nM).

For more information on sample accessioning, refer to *Sample Accessioning* and *Upload and Modify Samples* in the Getting Started section of the Clarity LIMS Core Functionality documentation.

You can assign samples to workflows automatically, using a routing script, or manually—from the Projects & Samples dashboard. Refer to *Assign and Process Samples* in the Clarity LIMS Core Functionality documentation.

Workflows, Protocols, and Steps

The Illumina NovaSeq 6000 Integration Package v2.4 includes two workflows:

- NovaSeq 6000 v2.2
- NovaSeq Validation Library Prep (NovaSeq 6000 v2.2)

The following table lists and describes the protocols and steps included in these workflows.

Table 259 NovaSeq 6000 Integration Package v2.4 Protocols and Steps

Protocol Name	Protocol Purpose	Protocol Steps
NovaSeq Validation Library Prep (NovaSeq 6000 v2.2) workflow		

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Protocol Name	Protocol Purpose	Protocol Steps
NovaSeq Validation Library Prep (NovaSeq 6000 v2.2)	Included for validation purposes only, this protocol models the library prep steps required to advance samples to the Run Format (NovaSeq 6000 v2.2) protocol. The protocol contains a single step: NovaSeq Validation Library Prep (NovaSeq 6000 v2.2). At the end of this step, a routing script sends the samples to the first step of the NovaSeq 6000 v2.2 workflow, which is Define Run Format (NovaSeq 6000 v2.2).	NovaSeq Validation Library Prep (NovaSeq 6000 v2.2)
	NovaSeq 6000 v2.2 workflow	
Run Format (NovaSeq 6000 v2.2)	Allows for the assignment of per sample values for Loading Workflow Type, Normalized Molarity, Flowcell Type, and Final Loading Concentration (pM). • Loading Workflow Type: Select from NovaSeq Standard or NovaSeq Xp. • Normalized Molarity: Enter a value for each sample. • Flowcell Type: Select from options S1, S2, S4, and SP. • Final Loading Concentration (pM): Select from the options 225 (PCR-free workflows) or 400 (Nano workflows). Alternatively, enter a different value. Compares the Normalized Molarity value of each sample with the Minimum Molarity value. Routing script sends samples to the NovaSeq Standard or NovaSeq Xp protocol, according to the selected Loading Workflow Type. Samples with Normalized Molarity less than Minimum Molarity are removed from the workflow.	Define Run Format (NovaSeq 6000 v2.2)
NovaSeq Standard (NovaSeq 6000 v2.2)	Samples are pooled and added to the library tube in preparation for the NovaSeq run. Sample sheet and run recipe files are generated. Routing script sends library tube to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.2) protocol.	Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.2) Dilute and Denature (NovaSeq 6000 v2.2)

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Protocol Name	Protocol Purpose	Protocol Steps
NovaSeq Xp (NovaSeq 6000 v2.2)	Samples are pooled and added to lanes on the NovaSeq flow cell. The flow cell type is determined by the option selected in the Define Run Format (NovaSeq 6000 v2.2) step. Sample sheet and run recipe files are generated. Samples are queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.2) protocol.	Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.2) Dilute, Denature, and ExAmp (NovaSeq 6000 v2.2) Load to Flowcell (NovaSeq 6000 v2.2)
AUTOMATED - NovaSeq Run (NovaSeq6000 v2.2)	All samples complete the workflow by going through this protocol. This protocol contains one fully automated step. Do not add samples to the Ice Bucket or start the step. The integration does this action automatically.	AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.2)

Validation Workflow

The NovaSeq Validation Library Prep (NovaSeq 6000 v2.2) workflow allows for validation of the system after installation is complete. For details, see *NovaSeq 6000 Integration v2.4 - Validation and Troubleshooting*.

Step Automations

The following table lists the step automations included in the NovaSeq 6000 v2.2 workflow. Automation details are provided in the sections following the table.

For details on the validation workflow, see *NovaSeq 6000 Integration v2.4 - Validation and Troubleshooting*.

Table 260 NovaSeq 6000 v2.2 Workflow Step Automations

Protocol	Step	Automation
1. Run Format (NovaSeq 6000 v2.2)	1. Define Run Format (NovaSeq 6000 v2.2)	• Routing Script • Set Next Step
2. NovaSeq Standard (NovaSeq 6000 v2.2)	1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.2)	• Calculate Volumes • Set Next Step • Validate Inputs Flowcell Type and Single Pool • Validate Unique Indexes Not used. This functionality is handled by the core Clarity LIMS configuration for pooling.
	2. Dilute and Denature (NovaSeq 6000 v2.2)	• Generate Sample Sheet and Run Recipe • Prepare Files for NovaSeq • Routing Script • Validate Library Tube Barcode • Validate Single Input
3. NovaSeq Xp (NovaSeq 6000 v2.2)	1. Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.2)	• Calculate Volumes • Set Next Step • Validate Inputs Flowcell Type and Single Pool • Validate Unique Indexes Not used. This functionality is handled by the core Clarity LIMS configuration for pooling.
	2. Dilute, Denature, and ExAmp (NovaSeq 6000 v2.2)	• Calculate Volumes • Set Next Step • Validate Inputs Flowcell Type
	3. Load to Flowcell (NovaSeq 6000 v2.2)	• Generate Sample Sheet and Run Recipe • Prepare Files for NovaSeq • Validate Flowcell Barcode • Validate Inputs and Selected Container
4. AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.2)	1. AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.2)	• Parse Run Parameters and Generate Link to Run Folder • Read InterOp Metrics • Set Next Steps • Update Lane number

Terminology

The Illumina NovaSeq 6000 Integration Package v2.4 supports Clarity LIMS v5 and later.

As of Clarity LIMS v5, the term user-defined field (UDF) has been replaced with custom field in the user interface. However, the API resource is still called UDF.

There are two types of custom fields:

- Master step fields—Configured on master steps. Master step fields only apply to the following:
  - The master step on which the fields are configured.
  - The steps derived from those master steps.
- Global fields—Configured on entities (eg, submitted sample, derived sample, measurement, etc.). Global fields apply to the entire Clarity LIMS system.

Protocol 1: Run Format (NovaSeq 6000 v2.2)

This protocol sets the Loading Workflow Type and allows you to choose the appropriate Flowcell Type and Final Loading Concentration(pM). At the end of the protocol, a routing script sends the normalized libraries to the NovaSeq Xp (NovaSeq 6000 v2.2) protocol.

This protocol contains one step: Define Run Format (NovaSeq 6000 v2.2).

Step input: NTP (normalized libraries)

Step output: None

Set Next Steps Automation

Automatically triggered on exit of the Record Details screen, this automation does the following actions:

- Sets the next step for samples to REMOVE with the `nextStep = ::REMOVE::` formula.
- Calculates the Minimum Molarity using the following formula:

```
input.::Minimum Molarity (nM):: = (5 * input.::Final Loading Concentration
(pM)::)/1000
```

- Checks Normalized Molarity value. For samples with no Normalized Molarity value (eg, an empty value, not including 0), the automation generates an error message stating that the field cannot be empty:

```
if (!input.hasValue(::Normalized Molarity (nM)::)) { fail(::The Normalized
Molarity cannot be empty.::) ; }
```

- Compares the Normalized Molarity value of each sample with the Minimum Molarity value.
- If Normalized Molarity value is lower than the Minimum Molarity value, sets the Loading Workflow Type of the sample to [Remove from workflow] and records a message in the Warning field:

```
else if (input.::Normalized Molarity (nM):: < input.::Minimum Molarity (nM)::) {
input.::Warning:: = ::The Normalized Molarity is too low.:: ; input.::Loading
Workflow Type:: = ::[Remove from workflow]:: ; } else { input.::Warning:: =
::n/a:: }
```

At this point, there are two options:

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- Correct the Normalized Molarity value on the Record Details screen. Also, edit the Loading Workflow Type field and set it to NovaSeq Standard or NovaSeq Xp, as applicable.
- Complete the protocol without correcting the Normalized Molarity value. In this case, those samples are removed from the workflow.

Routing Script Automation

Automatically triggered on exit of the step, this automation invokes the changeWorkflow script, which routes step inputs appropriately.

- Samples with Loading Workflow Type field value of NovaSeq Standard are routed to the NovaSeq 6000 v2.2 workflow and queued for the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.2) step.
- Samples with Loading Workflow Type field value of NovaSeq Xp are routed to the NovaSeq 6000 v2.2 workflow and queued for the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.2) step.

The default automation command line is as follows.

```
bash -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar
-u {username} -p {password} -i {stepURI:v2} -l {compoundOutputFileLuid0}
script:changeWorkflow \
\
--FIELD_NAME 'Loading Workflow Type' \
--FIELD_VALUE 'NovaSeq Standard' \
--WORKFLOW 'NovaSeq 6000 v2.2' \
--STEP 'Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.2)' \
--INPUTS_OR_OUTPUTS 'INPUTS' \
\
--FIELD_NAME 'Loading Workflow Type' \
--FIELD_VALUE 'NovaSeq Xp' \
--WORKFLOW 'NovaSeq 6000 v2.2' \
--STEP 'Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.2)' \
--INPUTS_OR_OUTPUTS 'INPUTS'"
```

Master Step Fields

There are four fields defined on the Define Run Format (NovaSeq 6000 v2.2) step.

The following table provides field configuration details.

Table 261 Define Run Format (NovaSeq 6000 v2.2) Master Step Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Comment	Multiline Text	• Required: No • Read Only: No	None
Instruction	Text	• Required: No • Read Only: Yes	Default: Add Flowcell Type and Loading Workflow Type below
Flowcell Type	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	Hidden
Loading Workflow Type	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	Hidden

Global Fields

The following table lists the global fields that are configured to display on the Define Run Format (NovaSeq 6000 v2.2) step.

Table 262 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Dropdown Items
Configured on Derived Sample			
Adjusted Per Sample Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Final Loading Concentration (pM)	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 225 • 400 • Decimal Places Displayed: 0
Flowcell Type	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• S1 • S2 • S4 • SP

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Field Name	Field Type	Options	Additional Options and Dropdown Items
Loading Workflow Type	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
Minimum Molarity (nM)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2
Normalized Molarity (nM)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2
Per Sample Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Warning	Text Dropdown	• Required: No • Read Only: Yes • Custom Entries: Yes	• The Normalized Molarity (nM) is too low. • Not applicable

User Interaction

1. On the Record Details screen in the Sample Details table, complete the following fields (values can vary across samples):
   - Loading Workflow Type: Select NovaSeq Standard or NovaSeq Xp from the drop-down list.
   - Flowcell Type: Select S1, S2, S4, or SP from the drop-down list.
   - Final Loading Concentration (pM): Select from the two preset options—225 (for PCR-free workflows) or 400 (for Nano workflows)—or enter a different value.
   - Normalized Molarity: Enter a value for each sample.
2. Select Next Steps.
   This selection triggers the Next Steps automation, which does the following actions:
   - Sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step successfully.
   - Calculates the Minimum Molarity.
   - Checks Normalized Molarity value. For samples with no Normalized Molarity value (ie, an empty value, not including 0), the automation generates an error message stating that the field cannot be empty.
   - Compares the Normalized Molarity value of each sample with the Minimum Molarity value.

3. On the Assign Next Steps screen, observe the following changes:
   - In the Sample Details table, the Next Step for all samples is prepopulated with Remove from workflow—regardless of the Loading Workflow Type.

     Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.

   - For samples whose Normalized Molarity value is lower than the Minimum Molarity value, the Loading Workflow Type is set to Remove from workflow and a message is recorded in the Warning field for the sample.
4. Complete one of the following options:
   - Return to the Record Details screen and adjust the Normalized Molarity value so that it equals or exceeds the Minimum Molarity value. Also, set the Loading Workflow Type to NovaSeq Standard.
   - Complete the protocol without correcting the Normalized Molarity value. In this case, the samples in question are removed from the Clarity LIMS workflow.
5. Select Finish Step.
   The Routing Script automation is triggered when:
   - Samples where Loading Workflow Type is set to Remove from workflow (eg, where Normalized Molarity value is lower than the Minimum Molarity) are removed from the Clarity LIMS workflow.
   - Samples where Loading Workflow Type is set to NovaSeq Standard are routed to the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.2) step. This step is the first of two steps in the NovaSeq Standard (NovaSeq 6000 v2.2) protocol.
   - Samples where Loading Workflow Type is set to NovaSeq Xp are routed to the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.2) step. This step is the first of three steps in the NovaSeq Xp (NovaSeq 6000 v2.2) protocol.

Protocol 2: NovaSeq Standard (NovaSeq 6000 v2.2)

Samples are routed to this protocol if the Loading Workflow Type value is set to NovaSeq Standard. Samples are pooled and added to a library tube in preparation for the NovaSeq run.

At the end of this protocol, a routing script sends the library tube to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.2) protocol.

This protocol contains two steps:

1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.2)
2. Dilute and Denature (NovaSeq 6000 v2.2)

Step 1: Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.2)

In this step, manually place libraries into a single pool. Resuspension buffer and reagents are added.

Only create one pool per step.

Step input: NTP (normalized libraries)

Step output: Bulk pool

The following automations are configured on the step:

- Calculate Volumes
- Set Next Step
- Validate Inputs Flowcell Type and Single Pool
- Validate Unique Indexes (Not used. This functionality is handled by the core Clarity LIMS configuration for pooling)

These automations are described in the order in which they are triggered at run time.

Validate Inputs Flowcell Type and Single Pool Automation

Automatically triggered on exit of the Pooling screen, this automation checks the following information:

- All samples in the pool have the same Flowcell Type assigned to them.
- Only one pool has been created.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/novaseq/novaseq-extensions.jar script:validate_flowcell_for_input_pools -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -validateSingleOutput true -poolType bulk
```

Calculate Volumes Automation

Due to the 4000-character limit, part of the script definition of Calculate Volumes has been moved into the Calculate Volumes Script field. If this part of the script needs to be modified, you can make the changes to the default value of the Calculate Volumes Script field. The parts of the script that were moved into the Calculate Volumes Script field are identified below.

Automatically triggered when the Calculate Volumes button on the Record Details screen is selected, this automation does the following actions:

- Calculates the number of samples in the pool:

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: + 1
```

- Sets the value of the Bulk Pool Volume (µl) field, based on the selected Flowcell Type. The following part of the automation is found in the Calculate Volumes Script field:

```
step.::PhiX Volume (ul):: = !step.hasValue(::% PhiX (2.5nM) Spike-In::) ? 0 : step.::% PhiX (2.5nM) Spike-In::;
if (input.::Flowcell Type:: == ::SP::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 100 + 30;
```


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```
step.::PhiX Volume (ul):: *= 0.8
};
if (input.::Flowcell Type:: == ::S1::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 100
+ 30;
step.::PhiX Volume (ul):: *= 0.8
};
if (input.::Flowcell Type:: == ::S2::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 150
+ 30;
step.::PhiX Volume (ul):: *= 1.1
};
if (input.::Flowcell Type:: == ::S4::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 310
+ 30;
step.::PhiX Volume (ul):: *= 2.1
};
if (step.::PhiX Volume (ul):: == 0) {
step.::PhiX Volume (ul):: = ::::
};
```

- Calculates the Per Sample Volume (µl) to be added to the pool. The following part of the automation is found in the Calculate Volumes Script field:

```
input.::Per Sample Volume (µl):: = ( ( (input.::Final Loading Concentration
(pM):: * 5 / 1000) / input.::Normalized Molarity (nM):: ) * step.::Bulk Pool
Volume (µl):: ) / step.::Number of Samples in Pool::
```

> To ensure accurate pipetting of each sample in a pool for sequencing, the Per Sample Volume (µl) value must be equal to or higher than the Minimum Per Sample Volume (µl). The default value is set at 5, which can be edited.
>
> Assuming the default Minimum Per Sample Volume (µl) value of 5, for a given batch:
>
> 1. If the smallest Per Sample Volume (µl) value is less than 5, Clarity LIMS automatically assigns a value of 5 to the Adjusted Per Sample Volume (µl) field.
> 2. Clarity LIMS then adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

- Calculates the Total Sample Volume (µl) field value:

```
step.::Total Sample Volume (µl):: = step.::Total Sample Volume (µl):: +
input.::Adjusted Per Sample Volume (µl)::
```

- If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) field value:

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```
if (step.::Total Sample Volume (µl):: >= step.::Bulk Pool Volume (µl)::)
{output.::RSB Volume (µl):: = 0} else {output.::RSB Volume (µl):: = step.::Bulk
Pool Volume (µl):: - step.::Total Sample Volume (µl)::} ;
```

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs:

```
output.::Flowcell Type::= input.::Flowcell Type::;output.::Loading Workflow
Type::= input.::Loading Workflow Type::;
```

- Sets the Volume of Pool to Denature (µl) value and calculates NaOH Volume (µl) and Tris-HCl Volume (µl) values, based on the Flowcell Type:

```
step.::PhiX Volume (ul):: = !step.hasValue('% PhiX (2.5nM) Spike-In') ? 0 :
step.'% PhiX (2.5nM) Spike-In';
if (input.::Flowcell Type:: == ::SP::) {
output.::Volume of Pool to Denature (ul):: = 100;
output.::NaOH Volume (ul):: = 25;
output.::Tris-HCl Volume (ul):: = 25;
}
if (input.::Flowcell Type:: == ::S1::) {
output.::Volume of Pool to Denature (ul):: = 100;
output.::NaOH Volume (ul):: = 25;
output.::Tris-HCl Volume (ul):: = 25;
}
if (input.::Flowcell Type:: == ::S2::) {
output.::Volume of Pool to Denature (ul):: = 150;
output.::NaOH Volume (ul):: = 37.50;
output.::Tris-HCl Volume (ul):: = 37.50;
}
if (input.::Flowcell Type:: == ::S4::) {
output.::Volume of Pool to Denature (ul):: = 310;
output.::NaOH Volume (ul):: = 77.50;
output.::Tris-HCl Volume (ul):: = 77.50;
}
```

- Uses the `NovaSeq_Standard_Bulk_Pool1.csv`, `NovaSeq_Standard_Bulk_Pool2.csv`, and `NovaSeq_Standard_Bulk_Pool3.csv` template files to generate a single CSV file containing information about the pool and the samples it contains. The generated file is available for download on the Step Setup screen of the following step: Dilute and Denature (NovaSeq 6000 v2.2).

```
script:driver_file_generator \ -t
/opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Standard_Bulk_Pool1.csv \
-o 1.csv \ script:driver_file_generator \ -t
/opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Standard_Bulk_Pool2.csv \
```

```
-o 2.csv \ script:driver_file_generator \ -t
/opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Standard_Bulk_Pool3.csv \
-o 3.csv \&& cat 1.csv 2.csv 3.csv > {compoundOutputFileLuid0}.csv
```

- Resets the Total Sample Volume (ul) and Number of Samples in Pool field values so that the automation is idempotent:

```
step.'Number of Samples in Pool' = step.'Number of Samples in Pool' - 1;
step.'Total Sample Volume (ul)' = step.'Total Sample Volume (ul)' -
input.'Adjusted Per Sample Volume (ul)'
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, the `nextStep = ::ADVANCE::` automation sets the next step for samples to ADVANCE, advancing them to the Dilute and Denature (NovaSeq 6000 v2.2) step in the protocol.

Master Step Fields

There are eight fields defined on the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.2) step.

The following table provides field configuration details.

Table 263 Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.2) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Drop-Down Items
% PhiX (2.5nM) Spike-In	Numeric	• Required: No • Read Only: No	• Range from 1 to 100 • Decimal Places Displayed: 0
Bulk Pool Volume (µl) (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2
Minimum Per Sample Volume (uL)	Numeric	• Required: Yes • Read Only: No	• Default: 5 • Decimal Places Displayed: 2

Field Name	Field Type	Options	Additional Options and Drop-Down Items
Number of Flowcells to Sequence	Numeric	• Required: Yes • Read Only: No	• Range From: 1 to 10 • Decimal Places Displayed: 0
Number of Samples in Pool (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Default: 0 • Decimal Places Displayed: 0
PhiX Volume (ul) (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2
Total Sample Volume (µl) (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Default: 0 • Decimal Places Displayed: 2
Calculate Volumes Script	Text	• Required: Yes • Read Only: Yes	• Default value provided in the drop-down section following the table Do not remove this field. It is used by the Calculate Volumes automation script.

Calculate Volumes Script

```
step.::PhiX Volume (ul):: = !step.hasValue(::% PhiX (2.5nM) Spike-In::) ? 0 :
step.::% PhiX (2.5nM) Spike-In::; if (input.::Flowcell Type:: == ::SP::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 100
+ 30; step.::PhiX Volume (ul):: *= 0.8 }; if (input.::Flowcell Type:: == ::S1::)
{ step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: *
100 + 30; step.::PhiX Volume (ul):: *= 0.8 }; if (input.::Flowcell Type:: ==
::S2::) { step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to
Sequence:: * 150 + 30; step.::PhiX Volume (ul):: *= 1.1 }; if (input.::Flowcell
Type:: == ::S4::) { step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells
to Sequence:: * 310 + 30; step.::PhiX Volume (ul):: *= 2.1 }; if (step.::PhiX
Volume (ul):: == 0) { step.::PhiX Volume (ul):: = :::: }; input.::Per Sample
Volume (ul):: = (((input.::Final Loading Concentration (pM):: * 5 / 1000) /
```

```
input.::Normalized Molarity (nM)::) * step.::Bulk Pool Volume (ul)::) /
step.::Number of Samples in Pool::
```

Global Fields

The following table lists the global fields that are configured to display on the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.2) step.

Table 264 Global Field Configuration

Field Name	Field Type	Options	Additional Options and Drop-Down Items
Configured on Derived Sample			
Flowcell Type	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• S1 • S2 • S4 • SP
Loading Workflow Type	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
NaOH Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
RSB Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Tris-HCl Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Volume of Pool to Denature (µl) (Used in Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.2) step only. Displays on Record Details screen and in the generated CSV file.)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0

User Interaction

1. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucket and select View Ice Bucket.
2. On the Ice Bucket screen, select Begin Work.
3. On the Pooling screen, create a pool as follows.
   a. Create a pool by dragging samples into the Pool Creator.
   b. Name the pool or accepts the default name (Pool #1).
   c. Select Record Detail.
   On exit from the Pooling screen, the Validate Inputs Flowcell Type and Single Pool is triggered. The automation checks that all samples in the pool have been assigned the same Flowcell Type and only one pool has been created.
4. On the Record Details screen in the Step Details area, complete the fields as follows.
   a. Enter the value for the Number of Flowcells to Sequence field.
   This value is used in volume calculations to make sure that the volumes are sufficient for the number of times the pool is sequenced.
   b. Enter the value for the Minimum Per Sample Volume (µl) field.
   The value in this field is used to calculate how much of each sample is included in the pool. The field is prepopulated with the configured default value (5 µl), but the value can be edited if necessary.
   c. If necessary, enter the value for the % PhiX (2.5 nM) Spike-In field.
   The value in this field is used to calculate the volume of PhiX v3 control to be included in the pool for the given percentage of spike-in. The field is optional and can be edited.
5. In the Sample Details table, select the pool icon to view details on the pool composition.
6. Select Calculate Volumes.
   This selection triggers the Calculate Volumes automation, which performs the following volume calculations based on the selected Flowcell Type:
   - Calculates Bulk Pool Volume (µl) value.
   - Calculates PhiX Volume (µl) value if % PhiX (2.5 nM) Spike-In has value.
   - Calculates the Per Sample Volume (µl) value to be added to the pool.
   - Calculates the Total Sample Volume (µl) value.
   - If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) value.
   - Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
   - Populates the Volume of Pool to Denature (µl), NaOH Volume (µl), and Tris-HCl Volume (µl) columns of the Sample Details table. (Values are set by a script and are not editable.)

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- Generates the Calculation File (CSV) and attaches it to the step. This file contains volume information about the pool and the individual samples it contains. Select the file to download it, then open it in Excel.

7. Select Next Steps.
   This selection triggers the Set Next Step automation. This automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol: Dilute and Denature (NovaSeq 6000 v2.2).
   On the Assign Next Steps screen, the next step is already set to Dilute and Denature (NovaSeq 6000 v2.2).
8. Select Finish Step.
   At the end of this step, the pool of samples automatically advances to the Dilute and Denature (NovaSeq 6000 v2.2) step.

Step 2: Dilute and Denature (NovaSeq 6000 v2.2)

In this step, pooled samples are denatured and diluted by the addition of NaOH, Tris-HCl, and Resuspension Buffer (RSB). Samples are manually placed into the library tube that is used in the NovaSeq run. In addition, this step generates the sample sheet file and the run recipe (*json file) needed to start the NovaSeq run. The step input is Bulk pool, and the step output is Library tube.

The following automations are configured on the step:

- Generate Sample Sheet and Run Recipe
- Prepare Files for NovaSeq
- Routing Script
- Validate Library Tube Barcode
- Validate Single Input

Validate Single Input Automation

Automatically triggered at the beginning of the step, the `script:validateSampleCount -min 1 -max 1` automation checks that there is only one container input to the step.

Validate Library Tube Barcode Automation

Automatically triggered on exit of the Placement screen, the following automation validates the library tube barcode to make sure it conforms to the barcode mask [A-Za-z]{2}[0-9]{7}-[A-Za-z]{3}:

```
if (!output.container.name.matches(::[A-Za-z]{2}[0-9]{7}-[A-Za-z]{3}::)) { fail (::Invalid Library Tube Barcode. Please verify and try again.::) }
```

Generate Sample Sheet and Run Recipe Automation

Automatically triggered when a button on the Record Details screen is selected, this automation does the following actions:

- Copies the Flowcell Type from the step input to the step output:

```
output.::Flowcell Type:: = input.::Flowcell Type::
```

- Copies the Flowcell Type from the step input to the Run Mode field (hidden):

```
step.::Run Mode:: = input.::Flowcell Type::
```

- Copies the Loading Workflow Type values from the step inputs to the step outputs:

```
output.::Loading Workflow Type:: = input.::Loading Workflow Type::;
```

- Validates the parameters entered on the Record Details screen:
  - Experiment Name can only contain alphanumeric, dash, or underscore characters. Spaces are not permitted.
  - When Workflow Type is No Index, Index Read 1 must be zero. For any other Workflow Type, Index Read 1 must be greater than zero.

```
if(!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) {
fail(::Experiment Name contains prohibited characters. Allowed characters are:
a-z, A-Z, 0-9, -, and _::)
}
if(step.::Workflow Type::== ::No Index::) {
if(step.::Index Read 1::!= 0) {
fail(::Index Read 1 must be 0 if the Workflow Type is No Index.::)
}
} else {
if(step.::Index Read 1::== 0) {
fail(::Index Read 1 must be greater than 0 if the Workflow Type is ::+
step.::Workflow Type::+ ::.::)
}
}
```

- When Paired End is True, Index Read 1 and Index Read 2 must be greater than zero.
- When Paired End is False, Index Read 2 must be zero, Reverse Complement Workflow must be false, UMI - Read 2 Length must not have a value, and UMI - Read 2 Start from Cycle must not have a value.

```
if (step.::Paired End::.toBoolean()) {
if (step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) {
fail(::Read 1 Cycles and Read 2 Cycles must be greater than 0 if Paired End is
True.::);
}
```

```
} else {
if (step.::Read 2 Cycles:: != 0) {
fail(::Read 2 Cycles must be 0 if Paired End is False.::);
}
if (step.hasValue(::UMI - Read 2 Length::) || step.hasValue(::UMI - Read 2 Start
From Cycle::)) {
fail(::UMI - Read 2 Length and UMI - Read 2 Start From Cycle cannot be defined
if Paired End is False.::);
}
}
```

- Validates allowed read cycles to be not greater than 151 if the Flowcell Type is not SP.

```
if (input.::Flowcell Type:: != ::SP:: && step.::Read 1 Cycles:: > 151) {
fail(::Read 1 Cycles must not be larger than 151 if it is not SPrime
Flowcell.::);
}
if (input.::Flowcell Type:: != ::SP:: && step.::Read 2 Cycles:: > 151) {
fail(::Read 2 Cycles must not be larger than 151 if it is not SPrime
Flowcell.::);
}
```

- Validates allowed value for UMI - Read 1 Length, UMI - Read 2 Length, UMI - Read 1 From Cycle, UMI - Read 2 From Cycle.

```
if (step.hasValue(::UMI - Read 1 Length::) && !step.hasValue(::UMI - Read 1
Start From Cycle::)) {
fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 1
Length is greater than 0.::);
}
if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 1
Start From Cycle::)) {
fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 1 Start From
Cycle is greater than 0.::);
}
if (step.hasValue(::UMI - Read 2 Length::) && !step.hasValue(::UMI - Read 2
Start From Cycle::)) {
fail(::UMI - Read 2 Start From Cycle must be greater than 0 if UMI - Read 2
Length is greater than 0.::);
}
if (!step.hasValue(::UMI - Read 2 Length::) && step.hasValue(::UMI - Read 2
Start From Cycle::)) {
fail(::UMI - Read 2 Length must be greater than 0 if UMI - Read 2 Start From
Cycle is greater than 0.::);
```


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```
}
if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 2
Length::)) {
fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 2 Length is
greater than 0.::);
}
if (!step.hasValue(::UMI - Read 1 Start From Cycle::) && step.hasValue(::UMI -
Read 2 Start From Cycle::)) {
fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 2
Start From Cycle is greater than 0.::);
}
```

- Sets the next step for samples to REMOVE.

```
nextStep = ::REMOVE::
```

- Generates the sample sheet and the run recipe file and attaches them to the step. For details on how the files are generated and their contents, see the *Sample Sheet and Run Recipe File Generation* on page 1137 section.

Prepare Files for NovaSeq Automation

Automatically triggered on exit of the Record Details screen, this automation invokes the `copy_attachments_to_network_folder` script, which is included in the `novaseq-remote-extensions.jar` file. This script places the generated sample sheet and run recipe (*json file) into the appropriate folder on the NAS, where the instrument uses them to start the run.

For more information, see the *Sample Sheet and Run Recipe File Generation* section.

Routing Script Automation

Automatically triggered on exit of the step, this automation invokes the changeWorkflow script, which routes step outputs to the NovaSeq 6000 v2.2 workflow, and queues them for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.2) step.

The default automation command line is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -u {username} -p {password} -i {stepURI:v2} -l
{compoundOutputFileLuid2} script:changeWorkflow \
\
--FIELD_NAME 'N/A' \
--FIELD_VALUE 'N/A' \
--WORKFLOW 'NovaSeq 6000 v2.2' \
--STEP 'AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.2)' \
--INPUTS_OR_OUTPUTS 'OUTPUTS'"
```

Master Step Fields

There are 19 fields defined on the Dilute and Denature (NovaSeq 6000 v2.2) step. These fields are required for sample sheet and JSON file generation.

The following table provides field configuration details.

Table 265 Dilute and Denature (NovaSeq 6000 v2.2) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Drop-Down Items
BaseSpace Sequence Hub Configuration	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• Not Used • Run Monitoring Only • Run Monitoring and Storage
Experiment Name	Text	• Required: Yes • Read Only: No	Not applicable
Index Read 1	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 0 • 6 • 8 • Range From: 0 to 20 • Decimal Places Displayed: 0
Index Read 2	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 0 • 6 • 8 • Range From: 0 to 20 • Decimal Places Displayed: 0
Paired End	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• True • False

Field Name	Field Type	Options	Additional Options and Drop-Down Items
Read 1 Cycles	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 251 • 151 • 101 • 51 • Range From: 1 to 251 • Decimal Places Displayed: 0
Read 2 Cycles	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 251 • 151 • 101 • 51 • Range From: 1 to 251 • Decimal Places Displayed: 0
Reverse Complement Workflow	Toggle Switch	• Required: Yes • Read Only: No	• Default: Yes
Run Mode (not displayed in user interface)	Text Dropdown	• Required: No • Read Only: Yes • Custom Entries: No	• S1 • S2 • S4
Sample Sheet Path	Text	• Required: No • Read Only: Yes	Not applicable
UMI — Read 1 Length	Numeric	• Required: No • Read Only: No	• Range From: 1 • Decimal Places Displayed: 0
UMI — Read 1 Start From Cycle	Numeric	• Required: No • Read Only: No	• Range From: 1 • Decimal Places Displayed: 0
UMI — Read 2 Length	Numeric	• Required: No • Read Only: No	• Range From: 1 • Decimal Places Displayed: 0
UMI — Read 2 Start From Cycle	Numeric	• Required: No • Read Only: No	• Range From: 1 • Decimal Places Displayed: 0

Field Name	Field Type	Options	Additional Options and Drop-Down Items
Use Custom Index Read 1 Primer	Toggle Switch	• Required: No • Read Only: No	• None Set
Use Custom Read 1 Primer	Toggle Switch	• Required: No • Read Only: No	• None Set
Use Custom Read 2 Primer	Toggle Switch	• Required: No • Read Only: No	• None Set
Workflow	Text	• Required: No • Read Only: Yes	• GenerateFASTQ
Workflow Type	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• No Index • Single Index • Dual Index

Global Step Fields

Table 8 lists the global step fields that are configured to display on the Dilute and Denature (NovaSeq 6000 v2.2) step.

Table 266 Table 8: Global UDF/Custom Field Configuration

Field Name	Field Type	Options	Additional Options and Drop-Down Items
Configured on derived sample:			
Flowcell Type	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• S1 • S2 • S4 • SP
Loading Workflow Type	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User interaction

1. On the Placement screen, move the pool as follows.
   a. Drag the pool into the library tube in the Placed Samples area.
   b. Scan or types the barcode of the library tube into the Library Tube field.
   c. Select Record Details.

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On exit of the Placement screen, the Validate Library Tube Barcode automation checks that the library tube barcode conforms to the barcode mask [A-Za-z]{2}[0-9]{7}-[A-Za-z]{3}. If not, an error message displays.

2. On the Record Details screen:

- The Reagent Lot Tracking section tracks the NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. Select from the active lots displayed in each drop-down list.

  To make reagent lots available for selection in the Reagent Lot Tracking section, configure and activate them. Do this step on the Configuration > Consumables screen.

- The fields displayed in the Step Details section are used to generate the sample sheet and run recipe files. Some of these fields are autopopulated and some must be completed manually (see Table 9 for details).

Table 267 Table 9: Fields Displayed on Record Details Screen of Dilute and Denature (NovaSeq 6000 v2.2) Step

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.
BaseSpace Sequence Hub Configuration	Select from preset options: Not Used, Run Monitoring Only, or Run Monitoring and Storage.
Workflow	Automatically populated with the preset GenerateFASTQ.
Workflow Type	Select from preset options: No Index, Single Index, and Dual Index.
Index Read 1	Select from preset option: 0, 6, or 8—or types a value between 0 and 20.
Index Read 2	Select from preset option: 0, 6, or 8—or types a value between 0 and 20.
Paired End	Select from preset options: True or False.
Read 1 Cycles	Select from preset options: 251, 151, 101, or 51—or types a value between 1 and 151.
Read 2 Cycles	Select from preset options: 251, 151, 101, or 51—or types a value between 1 and 151.
Use Custom Read 1 Primer	Select if applicable.
Use Custom Read 2 Primer	Select if applicable.

Field	Value
Use Custom Index Read 1 Primer	Select if applicable.
Reverse Complement Workflow	Select Yes (by default) for v1.5 reagents in NovaSeq Control Software v1.7 and onwards. Select No for v1 reagents.
UMI — Read 1 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 1. Leave blank if not applicable.
UMI — Read 2 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 2. Leave blank if not applicable.
UMI — Read 1 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.
UMI — Read 2 Start From Cycle	Enter the cycle number that Read 2 starts from. Leave blank if not applicable.
Sample Sheet Path	Automatically populated after the sample sheet has been generated.

3. Select Generate Sample Sheet and Run Recipe.
   This selection triggers the automation script, which does the following actions:
   - Generates the sample sheet and JSON files and attaches them to the placeholders in the Files area of the Record Details screen.
   - Populates the Sample Sheet Path field with the path to the sample sheet file.
   - Sets the value of the next step to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step successfully.
4. Select Next Steps.
   This selection triggers the Prepare Files for NovaSeq automation, which copies the sample sheet and run recipe files to the location specified during installation.
5. On the Assign Next Steps screen, make sure the Next Step for all samples is prepopulated with Remove from workflow.

   Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.

6. Select Finish Step.
   This will trigger the Routing Script automation and route samples to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.2) protocol. In Lab View, the pool of samples is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.2) step.

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At this point in the workflow, the user interaction ends. The integration automatically starts the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.2) step and data from the run is parsed back into Clarity LIMS.

Protocol 3: NovaSeq Xp (NovaSeq 6000 v2.2)

Samples are routed to this protocol if the Loading Workflow Type value is set to NovaSeq Xp.

Samples are pooled and added to lanes on the NovaSeq flow cell type selected in the Define Run Format (NovaSeq 6000 v2.2) step.

At the end of the protocol, a routing script sends the flow cell to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.2) protocol.

This protocol contains three steps:

1. Step 1: Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.2)
2. Step 2: Dilute, Denature, and ExAmp (NovaSeq 6000 v2.2)
3. Step 3: Load to Flowcell (NovaSeq 6000 v2.2)

Step 1: Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.2)

In this step, the libraries are manually placed into a pool. The step input is NTP (normalized libraries) and the step output is Bulk pool.

Only create one pool per step.

The following automation is configured on the step:

- Calculate Volumes
- Set Next Step
- Validate Inputs Flowcell Type and Single Pool
- Validate Unique Indexes (Not used. This functionality is handled by the core Clarity LIMS configuration for pooling.)

These automations are described in the order in which they are triggered at run time.

Validate Inputs Flowcell Type and Single Pool

Automatically triggered on exit of the Pooling screen, this automation checks the following information:

- All samples in the pool have the same Flowcell Type assigned to them.
- Only one pool has been created.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/novaseq/novaseq-
extensions.jar script:validate_flowcell_for_input_pools -i {stepURI:v2} -u
{username} -p {password} -l {compoundOutputFileLuid1} -validateSingleOutput true
```


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Calculate Volumes

Automatically triggered when the Calculate Volumes button on the Record Details screen is selected, this automation:

- Calculates the number of samples in the pool:

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: + 1
```

- Sets the value of the Bulk Pool Volume (µl) and PhiX Volume (µl) field, based on the selected Flowcell Type:

```
step.::PhiX Volume (µl):: = !step.hasValue(::% PhiX (0.25nM) Spike-In::) ? 0 :
step.::% PhiX (0.25nM) Spike-In::;
if (input.::Flowcell Type:: == ::SP::) {
step.::Bulk Pool Volume (µl):: = step.'Number of Lanes to Sequence' * 18 + 30;
step.::PhiX Volume (µl):: *= 1.9;
}
if (input.::Flowcell Type:: == ::S1::) {
step.::Bulk Pool Volume (µl):: = step.'Number of Lanes to Sequence' * 18 + 30;
step.::PhiX Volume (µl):: *= 1.9;
}
if (input.::Flowcell Type:: == ::S2::) {
step.::Bulk Pool Volume (µl):: = step.'Number of Lanes to Sequence' * 22 + 30;
step.::PhiX Volume (µl):: *= 1.9;
}
if (input.::Flowcell Type:: == ::S4::) {
step.::Bulk Pool Volume (µl):: = step.'Number of Lanes to Sequence' * 30 + 30;
step.::PhiX Volume (µl):: *= 2.2;
}
if (step.::PhiX Volume (µl):: == 0) {
step.::PhiX Volume (µl):: = '';
}
```

- Calculates the Per Sample Volume (µl) to be added to the pool:

```
input.::Per Sample Volume (µl)::= (((input.::Final Loading Concentration (pM)::*
5/ 1000) / input.::Normalized Molarity (nM)::) * step.::Bulk Pool Volume (µl)::)
/ step.::Number of Samples in Pool::
```


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> To ensure accurate pipetting of each sample in a pool for sequencing, the Per Sample Volume (µl) value must be equal to or higher than the Minimum Per Sample Volume (µl). The default value set at 5 and can be edited.
>
> Assuming the default Minimum Per Sample Volume (µl) value of 5, for a given batch:
>
> 1. If the smallest Per Sample Volume (µl) value is less than 5, Clarity LIMS automatically assigns a value of 5 to the sample's Adjusted Per Sample Volume (µl) field.
> 2. Clarity LIMS then adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

- Calculates the Total Sample Volume (µl) field value:

```
step.::Total Sample Volume (µl):: = step.::Total Sample Volume (µl):: +
input.::Adjusted Per Sample Volume (µl)::
```

- If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) field value:

```
if (step.::Total Sample Volume (µl):: >= step.::Bulk Pool Volume (µl)::)
{output.::RSB Volume (µl):: = 0} else {output.::RSB Volume (µl):: = step.::Bulk
Pool Volume (µl):: - step.::Total Sample Volume (µl)::} ;
```

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs:

```
output.::Flowcell Type::= input.::Flowcell Type::;output.::Loading Workflow
Type::= input.::Loading Workflow Type::;
```

- Uses the `NovaSeq_Xp_Bulk_Pool.csv` and `NovaSeq_Xp_Bulk_Pool2.csv` template files to generate a single CSV file containing information about the bulk pool and the samples it contains. The generated file is available for download on the Step Setup screen of the following step—Dilute, Denature, and ExAmp (NovaSeq 6000 v2.2).
- Resets the Total Sample Volume (ul) and Number of Samples in Pool field values so that the automation is idempotent:

```
step.'Number of Samples in Pool' = step.'Number of Samples in Pool' - 1;
step.'Total Sample Volume (ul)' = step.'Total Sample Volume (ul)' -
input.'Adjusted Per Sample Volume (ul)'
```

Set Next Step

Automatically triggered on exit of the Record Details screen, this automation does the following actions:

- Copies the Flowcell Type values from the step inputs to the step outputs.
- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ; nextStep = ::ADVANCE::
```


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Master Step Fields

There are seven fields defined on the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.2) step.

The following table provides field configuration details.

Table 268 Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.2) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Drop-Down Items
% PhiX (0.25nM) Spike-In	Numeric	• Required: No • Read Only: No	• Range: 0 to 100 • Decimal Places Displayed: 0
Bulk Pool Volume (µl) (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2
Minimum Per Sample Volume (µl)	Numeric	• Required: Yes • Read Only: No	• Default: 5 • Decimal Places Displayed: 2
Number of Lanes to Sequence	Numeric	• Required: Yes • Read Only: No	• Decimal Places Displayed: 0
Number of Sample in Pool (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Default: 0 • Decimal Places Displayed: 0
PhiX Volume (µl) (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2
Total Sample Volume (µl) (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Default: 0 • Decimal Places Displayed: 0

Global Fields

The following table lists the global fields that are configured to display on the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.2) step.

Table 269 Global Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Configured on Derived Sample			
RSB Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Flowcell Type	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• S1 • S2 • S4 • SP
Loading Workflow Type	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User Interaction

1. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucket and select View Ice Bucket.
2. On the Ice Bucket screen, select Begin Work.
3. On the Pooling screen, create a pool as follows.
   a. Drag samples into the Pool Creator to create a pool.
   b. Name the pool or accepts the default name Pool #1.
   c. Select Record Details.

   On exit from the Pooling screen, the Validate Inputs Flowcell Type and Single Pool is triggered. The automation checks that all samples in the pool have been assigned the same Flowcell Type and only one pool has been created.
4. On the Record Details screen in the Step Details area, complete the fields as follows.
   a. Enter the value for the Number of Lanes to Sequence field.
      This value is used in volume calculations to make sure that the volumes are sufficient for the number of times the pool is sequenced.
   b. Enter the value for the Minimum Per Sample Volume (µl) field.
      The value in this field is used to calculate how much of each sample is included in the pool. The field is prepopulated with the configured default value (5 µl), but the value can be edited if necessary.
   c. If necessary, enter the value for the % PhiX (0.25 nM) Spike-In field.

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The value in this field is used to calculate the volume of PhiX v3 control to be included in the pool for the given percentage of spike-in. The field is optional and can be edited.

5. In the Sample Details table, select the pool icon to view details on the pool composition.
6. Select Calculate Volumes.
   This selection triggers the Calculate Volumes automation, which performs the following volume calculations based on the selected Flowcell Type:
   - Calculates Bulk Pool Volume (µl) value.
   - If % PhiX (0.25nM) Spike-In has a value, calculates the PhiX Volume (µl) value.
   - Calculates the Per Sample Volume (µl) value to be added to the pool.
   - Calculates the Total Sample Volume (µl) value.
   - If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) value.
   - Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
   - Generates the Calculation File (*csv) and attaches it to the step. This file contains volume information about the samples and RSB buffer to add to the pool. Select the file to download it, then open it in Excel.
7. Select Next Steps.
   This selection triggers the Set Next Step automation. This automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol—Dilute, Denature, and ExAmp (NovaSeq 6000 v2.2).
   On the Assign Next Steps screen, the next step is already set to Dilute, Denature, and ExAmp (NovaSeq 6000 v2.2).
8. Select Finish Step.
   At the end of this step, the pool of samples automatically advances to the Dilute, Denature, and ExAmp (NovaSeq 6000 v2.2) step.

Step 2: Dilute, Denature, and ExAmp (NovaSeq 6000 v2.2)

In this step, pooled samples are denatured and diluted by the addition of DPX, NaOH, Tris-HCl, and RSB. Manually create working pools based on the number of lanes you want to sequence. The step input is Bulk pool and the step output is Working pool. Working pool is a variable number for how many working pools need to be created per bulk pool.

The following automations are configured on the step:

- Calculate Volumes
- Set Next Step
- Validate Inputs Flowcell Type

These automations are described in the order in which they are triggered at run time.

Validate Inputs Flowcell Type Automation

Automatically triggered at the beginning of the step, this automation invokes the `validate_flowcell_for_input_pools` script. This script does the following actions:

- Checks the inputs to the step and validates that the Flowcell Type field has been set to a valid value (SP, S1, S2, or S4). The automation also checks that all inputs have the same value.
- Validates that the number of outputs matches the number of lanes on the selected flow cell type. If validation fails, an error message state that the number of working pools does not match the number of lanes available on the flow cell.

```
bash -l -c "/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/novaseq/novaseq-extensions.jar script:validate_
flowcell_for_input_pools -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1}"
```

Calculate Volumes Automation

Automatically triggered on entry to of the Record Details screen, this automation sets the value of the following fields, based on the Flowcell Type:

- Bp Aliquot Volume (µl)
- NaOH Volume (µl)
- Tris-HCl Volume (µl)
- DPX1 Volume (µl)
- DPX2 Volume (µl)
- DPX3 Volume (µl)
- Mastermix per Lane (µl)

```
if (input.::Flowcell Type:: == ::SP::) {
output.::BP Aliquot Volume (ul):: = 18;
output.::NaOH Volume (ul):: = 4.50;
output.::Tris-HCl Volume (ul):: = 4.50;
step.::DPX1 Volume (ul):: = 126;
step.::DPX2 Volume (ul):: = 18;
step.::DPX3 Volume (ul):: = 66;
output.::Mastermix per Lane (ul):: = 63
};
if (input.::Flowcell Type:: == ::S1::) {
output.::bp Aliquot Volume (µl):: = 18;
output.::NaOH Volume (µl):: = 4.50;
output.::Tris-HCl Volume (µl):: = 4.50;
```


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```
step.::DPX1 Volume (µl):: = 126;
step.::DPX2 Volume (µl):: = 18;
step.::DPX3 Volume (µl):: = 66;
output.::Mastermix per Lane (µl):: = 63
};
if (input.::Flowcell Type:: == ::S2::) {
output.::bp Aliquot Volume (µl):: = 22;
output.::NaOH Volume (µl):: = 5.50;
output.::Tris-HCl Volume (µl):: = 5.50;
step.::DPX1 Volume (µl):: = 126;
step.::DPX2 Volume (µl):: = 18;
step.::DPX3 Volume (µl):: = 66;
output.::Mastermix per Lane (µl):: = 77
};
if (input.::Flowcell Type:: == ::S4::) {
output.::bp Aliquot Volume (µl):: = 30;
output.::NaOH Volume (µl):: = 7.50;
output.::Tris-HCl Volume (µl):: = 7.50;
step.::DPX1 Volume (µl):: = 315;
step.::DPX2 Volume (µl):: = 45;
step.::DPX3 Volume (µl):: = 165;
output.::Mastermix per Lane (µl):: = 105
};
```

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs:

```
output.::Flowcell Type::= input.::Flowcell Type::;
output.::Loading Workflow Type::= input.::Loading Workflow Type::;
```

- Uses the `NovaSeq_Xp_Working_Pool.csv` and `NovaSeq_Xp_Working_Pool2.csv` template files to generate a single CSV file containing information about the DPX volume and the volume of bp Aliquot, Mastermix, NaOH, and Tris-HCl to add per working pool. The generated file is available for download on the Step Setup screen of the following step—Load to Flowcell (NovaSeq 6000 v2.2).

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation:

- Copies the Flowcell Type values from the step inputs to the step outputs.
- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ; nextStep = ::ADVANCE::
```


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Master Step Fields

There are three fields defined on the Dilute, Denature, and ExAmp (NovaSeq 6000 v2.2) step. These field values are set by a script and are not editable while running the step.

The following table provides field configuration details.

Table 270 Dilute, Denature, and ExAmp (NovaSeq 6000 v2.2) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Drop-Down Items
DPX1 Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0
DPX2 Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0
DPX3 Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0

Global Fields

The following table lists the global fields that are configured to display on the Dilute, Denature, and ExAmp (NovaSeq 6000 v2.2) step.

Table 271 Global Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Configured on Derived Sample			
Bp Aliquot Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0
Flowcell Type	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• SP • S1 • S2 • S4
Loading Workflow Type	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Mastermix per Lane (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0
NaOH Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Tris-HCl Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2

User Interaction

1. On the Queue screen, add the pool to the Ice Bucket and select View Ice Bucket.
2. On the Ice Bucket screen, set the number of derivatives to create (they are placed into the flow cell lanes) and select Begin Work.
   On entry to the Record Details screen, the Calculate Volumes automation is triggered.
   This automation sets the following values based on the selected Flowcell Type (calculation details are provided in the previous Calculate Volumes Automation section):
   - Bp Aliquot Volume (µl)
   - NaOH Volume (µl)
   - Tris-HCl Volume (µl)
   - DPX1 Volume (µl)
   - DPX2 Volume (µl)
   - DPX3 Volume (µl)
   - Mastermix per Lane (µl)

   The automation also generates the Calculation File (*csv) and attaches it to the step. This file contains information about the DPX Mastermix volume and the volume of Mastermix, NaOH, and Tris-HCl to add per working pool. Select the file to download it, then open it in Excel.
3. On the Record Details screen, the Reagent Lot Tracking section tracks the DPX1, DPX2, DPX3, NaOH, RSB, and Tris-HCl reagent lots. Select from the active lots displayed in each drop-down list.

   > To make reagent lots available for selection in the Reagent Lot Tracking section, configure and activate them. Do this step on the Configuration > Consumables screen.

4. In the Step Details area, the DPX1, DPX2, and DPX3 reagent volume values are already populated. (These values are set by a script and are not editable while running the step.)
5. In the Sample Details table, select the pool icon to view details on the working pool composition.

- The bp Aliquot, Mastermix per lane, NaOH, and Tris-HCl volume values for each working pool are already populated. (These values are set by a script and are not editable while running the step.)
- The working pool number is appended to the bulk pool name. This feature helps quickly identify which working pools are derived from the same bulk pool.

6. In the Files area, select the Calculation File (*csv) file to download it. Open the file in Excel to view information about the DPX Mastermix volume and the volume of Mastermix, NaOH, and Tris-HCl to add per working pool.
7. Select Next Steps to trigger the Set Next Step automation.
   On the Assign Next Steps screen, the next step is already set to Load to Flowcell (NovaSeq 6000 v2.2).
8. Select Finish Step.

Step 3: Load to Flowcell (NovaSeq 6000 v2.2)

In this step, scan the flow cell barcode into Clarity LIMS, then manually place the working pools into the lanes of the flow cell that is used in the NovaSeq run. The step input is Working pool and the step output is Flow cell (output containers: S1 and S2 with 2 lanes, and S4 with 4 lanes).

The following automations are configured on this step:

- Generate Sample Sheet and Run Recipe
- Prepare Files for NovaSeq
- Validate Flowcell Barcode
- Validate Inputs and Selected Container

These automations are described in the order in which they are triggered at run time.

Validate Inputs and Selected Container Automation

Automatically triggered at the beginning of the step, this automation invokes the `validate_flowcell_for_input_pools` and `validateSelectedContainer` scripts. These scripts validate the step inputs and the container selected, as follows.

- Check that the Flowcell Type field has been set to a valid value (S1, S2, S4, or SP) and that all inputs have the same value.
- Check that the number of outputs matches the number of lanes on the selected flow cell type. If validation fails, an error message states that the number of working pools does not match the number of lanes available on the flow cell. The following table defines the number of samples allowed:

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Flowcell Type	Number of Working Pools
SP	2
S1	2
S2	2
S4	4

- Check that the container type selected on entry to the Placement screen matches the value in the Flowcell Type field. If validation fails, an error message displays.

Validate Flowcell Barcode

Automatically triggered on exit of the Placement screen, this automation validates the flow cell barcode scanned into Clarity LIMS, using the following logic:

```
if (input.::Flowcell Type:: == ::SP:: && !output.container.name.matches(::[A-Za-z0-9]{5}DRX[XY2357]::)) {
fail(::Invalid Flowcell Barcode. Please verify and try again.::)
};
if (input.::Flowcell Type:: == ::S1:: && !output.container.name.matches(::[A-Za-z0-9]{5}DRX[XY2357]::)) {
fail(::Invalid Flowcell Barcode. Please verify and try again.::)
};
if (input.::Flowcell Type:: == ::S2:: && !output.container.name.matches(::[A-Za-z0-9]{5}DMX[XY2357]::)) {
fail(::Invalid Flowcell Barcode. Please verify and try again.::)
};
if (input.::Flowcell Type:: == ::S4:: && !output.container.name.matches(::[A-Za-z0-9]{5}DSX[XY2357]::)) {
fail(::Invalid Flowcell Barcode. Please verify and try again.::)
};
```

Generate Sample Sheet and Run Recipe

Automatically triggered when a button on the Record Details screen is selected, this automation:

- Copies the Flowcell Type from the step input to the Run Mode field (hidden):

```
step.::Run Mode:: = input.::Flowcell Type::
```

- Validates the parameters entered on the Record Details screen:
  - Experiment Name can only contain alphanumeric, dash, or underscore characters. Spaces are not permitted.

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- When Workflow Type is No Index, Index Read 1 must be zero. For any other Workflow Type, Index Read 1 must be greater than zero.

```
if(!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) {
fail(::Experiment Name contains prohibited characters. Allowed characters are:
a-z, A-Z, 0-9, -, and _::)
};
if(step.::Workflow Type::== ::No Index::) {
if(step.::Index Read 1::!= 0) {
fail(::Index Read 1 must be 0 if the Workflow Type is No Index.::)
}
} else{
if(step.::Index Read 1::== 0) {
fail(::Index Read 1 must be greater than 0 if the Workflow Type is ::+
step.::Workflow Type::+ ::.::)
}
};
```

- When Paired End is True, Index Read 1 and Index Read 2 must be greater than zero.
- When Paired End = False, Index Read 2 must be zero. Reverse Complement Workflow must be false. UMI - Read 2 Length and UMI - Read 2 Start from Cycle must not have a value.

```
if (step.::Paired End::.toBoolean()) {if (step.::Read 1 Cycles:: == 0 ||
step.::Read 2 Cycles:: == 0) {fail(::Read 1 Cycles and Read 2 Cycles must be
greater than 0 if Paired End is True.::);}} else {if (step.::Read 2 Cycles:: !=
0) {fail(::Read 2 Cycles must be 0 if Paired End is False.::);}
if (step.hasValue(::UMI - Read 2 Length::) || step.hasValue(::UMI - Read 2 Start
From Cycle::)) {fail(::UMI - Read 2 Length and UMI - Read 2 Start From Cycle
cannot be defined if Paired End is False.::);}}

```

- Validates allowed read cycles to be not greater than 151 if the Flowcell Type is not SP.

```
if (input.::Flowcell Type:: != ::SP:: && step.::Read 1 Cycles:: > 151) {
fail(::Read 1 Cycles must not be larger than 151 if it is not SPrime
Flowcell.::);
}
if (input.::Flowcell Type:: != ::SP:: && step.::Read 2 Cycles:: > 151) {
fail(::Read 2 Cycles must not be larger than 151 if it is not SPrime
Flowcell.::);
}
```

- Validates allowed value for UMI - Read 1 Length, UMI - Read 2 Length, UMI - Read 1 From Cycle, UMI - Read 2 From Cycle.

```
if (step.hasValue(::UMI - Read 1 Length::) && !step.hasValue(::UMI - Read 1
Start From Cycle::)) {
fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 1
Length is greater than 0.::);
}
if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 1
Start From Cycle::)) {
fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 1 Start From
Cycle is greater than 0.::);
}
if (step.hasValue(::UMI - Read 2 Length::) && !step.hasValue(::UMI - Read 2
Start From Cycle::)) {
fail(::UMI - Read 2 Start From Cycle must be greater than 0 if UMI - Read 2
Length is greater than 0.::);
}
if (!step.hasValue(::UMI - Read 2 Length::) && step.hasValue(::UMI - Read 2
Start From Cycle::)) {
fail(::UMI - Read 2 Length must be greater than 0 if UMI - Read 2 Start From
Cycle is greater than 0.::);
}
if (!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 2
Length::)) {
fail(::UMI - Read 1 Length must be greater than 0 if UMI - Read 2 Length is
greater than 0.::);
}
if (!step.hasValue(::UMI - Read 1 Start From Cycle::) && step.hasValue(::UMI -
Read 2 Start From Cycle::)) {
fail(::UMI - Read 1 Start From Cycle must be greater than 0 if UMI - Read 2
Start From Cycle is greater than 0.::);
}
```

  - Copies the Flowcell Type value from the step inputs to the step outputs:

```
output.::Flowcell Type::= input.::Flowcell Type::;
```

  - Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol—AUTOMATED - Run (NovaSeq 6000) v2.2.

```
nextStep = ::ADVANCE::
```

  - Generates the sample sheet and the run recipe file and attaches them to the step. For details on how the files are generated, and their contents, see the *Sample Sheet and Run Recipe File Generation* section.

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Prepare Files for NovaSeq

Automatically triggered on exit of the Record Details screen, this automation invokes the `copy_attachments_to_network_folder` script, which is included in the `novaseq-remote-extensions.jar` file. This script places the generated sample sheet and run recipe (*json file) into the appropriate folder on the NAS, where the instrument uses them to start the run.

For more information, see the *Sample Sheet and Run Recipe File Generation* section.

Master Step Fields

There are 20 fields defined on the Load to Flowcell (NovaSeq 6000 v2.2) step. The following table provides field configuration details.

Table 272 Load to Flowcell (NovaSeq 6000 v2.2) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Drop-Down Items
BaseSpace Sequence Hub Configuration	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• Not Used • Run Monitoring Only • Run Monitoring and Storage
Experiment Name	Text	• Required: Yes • Read Only: No	Not applicable
Index Read 1	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 0 • 6 • 8 • Range From: 0 to 20 • Decimal Places Displayed: 0
Index Read 2	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 0 • 6 • 8 • Range From: 0 to 20 • Decimal Places Displayed: 0

Field Name	Field Type	Options	Additional Options and Drop-Down Items
Library Tube Barcode	Text	• Required: Yes • Read Only: No	Not applicable
Paired End	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• True • False
Read 1 Cycles	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 251 • 151 • 101 • 51 • Range From: 1 to 251 • Decimal Places Displayed: 0
Read 2 Cycles	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 251 • 151 • 101 • 51 • Range From: 1 to 251 • Decimal Places Displayed: 0
Reverse Complement Workflow	Toggle Switch	• Required: Yes • Read Only: No	• Default: Yes
Run Mode (not displayed in user interface)	Text Dropdown	• Required: No • Read Only: Yes • Custom Entries: No	• S1 • S2 • S4 • SP
Sample Sheet Path	Text	• Required: No • Read Only: Yes	Not applicable
UMI—Read 1 Length	Numeric	• Required: No • Read Only: No	• Range From: 1 • Decimal Places Displayed: 0
UMI—Read 1 Start From Cycle	Numeric	• Required: No • Read Only: No	• Range From: 1 • Decimal Places Displayed: 0

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Field Name	Field Type	Options	Additional Options and Drop-Down Items
UMI—Read 2 Length	Numeric	• Required: No • Read Only: No	• Range From: 1 • Decimal Places Displayed: 0
UMI—Read 2 Start From Cycle	Numeric	• Required: No • Read Only: No	• Range From: 1 • Decimal Places Displayed: 0
Use Custom Index Read 1 Primer	Toggle Switch	• Required: No • Read Only: No	• Default: No
Use Custom Read 1 Primer (For Clarity LIMS v5.0.5 and later, see Configuration Update documentation.)	Toggle Switch	• Required: No • Read Only: No	• Default: No
Use Custom Read 2 Primer (For Clarity LIMS v5.0.5 and later, see Configuration Update documentation.)	Toggle Switch	• Required: No • Read Only: No	• Default: No
Workflow	Text	• Required: No • Read Only: Yes	• GenerateFASTQ
Workflow Type	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• No Index • Single Index • Dual Index

Global Fields

The following table lists the global fields that are configured to display on the Load to Flowcell (NovaSeq 6000 v2.2) step.

Field Name	Field Type	Options	Additional Options and Drop-Down Items
Configured on Derived Sample			
Flowcell Type	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• S1 • S2 • S4 • SP

User Interaction

1. On the Ice Bucket screen, select the appropriate flow cell type from the Destination Container drop-down list in the Container Options panel.
2. Select Begin Work.
3. On the Placement screen, move the pools as follows.
   a. Drag the pools from the left of the screen over into the Placed Samples area on the right.
   b. Scan or type the barcode of the flow cell into the flow cell field.
   c. Select Record Details.
   On exit of the Placement screen, the Validate Flowcell Barcode automation validates the container barcode (described previously). The fields displayed on the Record Details screen are used to generate the sample sheet and run recipe files. Some of these fields are autopopulated and some must be completed manually.

Table 273 Fields Displayed on Record Details Screen of Load to Flowcell (NovaSeq 6000 v2.2) Step

Field	Value
Experiment Name	Enter the name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.
BaseSpace Sequence Hub Configuration	Select from preset options: Not Used, Run Monitoring Only, or Run Monitoring and Storage.
Workflow	Automatically populated with preset—GenerateFASTQ.
Workflow Type	Select from preset options: No Index, Single Index, or Dual Index.

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Field	Value
Index Read 1	Select from preset option: 0, 6, or 8—or types a value between 0 and 20.
Index Read 2	Select from preset options: 0, 6, 8—or type a value between 0 and 20.
Paired End	Select from preset options: True or False.
Read 1 Cycles	Select from preset options: 251, 151, 101, or 51—or type a value between 1 and 251.
Read 2 Cycles	Select from preset options: 251, 151, 101, or 51—or type a value between 1 and 251.
Use Custom Read 1 Primer	Select, if applicable.
Use Custom Read 2 Primer	Select, if applicable.
Use Custom Index Read 1 Primer	Select, if applicable.
Reverse Complement Workflow	Select Yes (by default) for v1.5 reagents in NovaSeq Control Software v1.7 and onwards. Select No for v1 reagents.
UMI - Read 1 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 1. Leave blank if not applicable.
UMI - Read 2 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 2. Leave blank if not applicable.
UMI - Read 1 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.
UMI - Read 2 Start From Cycle	Enter the cycle number that Read 2 starts from. Leave blank if not applicable.
Sample Sheet Path	Automatically populated after the sample sheet has been generated.
Library Tube Barcode	Scan the library tube barcode.

4. On the Record Details screen, select Generate Sample Sheet and Run Recipe.
   This selection triggers the automation script, which does the following actions:
   - Generates the sample sheet and JSON files and attaches them to the placeholders in the Files area of the Record Details screen.
   - Populates the Sample Sheet Path field with the path to the sample sheet file.

5. Select Next Steps to trigger the Prepare Files for NovaSeq automation, which copies the sample sheet and run recipe files to the location specified during installation.
   On the Assign Next Steps screen, the Next Step for all samples is prepopulated with Mark protocol as complete.
6. Select Finish Step.
   In Lab View, the pool of samples is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.2) step.

At this point in the workflow, the user interaction ends. The integration automatically starts the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.2) step and data from the run is parsed back into Clarity LIMS.

Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.2)

This final protocol contains one fully automated step, AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.2).

Step 1: AUTOMATED - NovaSeq Run (NovaSeq 6000) 2.2

Step input: Library tube from NovaSeq Standard protocol or NovaSeq Xp protocol.

Step output: Measurement.

Do not start this step. This is a fully automated step. The sequencing service does not update samples correctly if they have been manually started.

In this step, pooled samples are sequenced on the NovaSeq instrument. The run data network folder is monitored by the sequencing service and automation worker to determine when the sequencing run begins and ends.

1. When a run is initiated on the NovaSeq instrument, the NovaSeq Control Software (NVCS) looks for the sample sheet and run recipe (*.json file) on the shared network drive.
   - The NVCS copies the sample sheet to the run directory.
   - The NVCS uses the run recipe to start the sequencing run.
2. When the run starts:
   - The instrument software creates a new run folder (named < libraryID >) on the shared network drive and copies the following files into that folder:
     - RunParameters.xml
     - RunInfo.xml
     - LIMS/ <libraryID>.json file (run recipe)
   - The Real-Time Analysis v3 (RTA3) software creates the InterOp folder.
3. The sequencing service detects the presence of the RunParameters, RunInfo, and JSON files and starts the AUTOMATED- NovaSeq Run (NovaSeq 6000 v2.2) step in Clarity LIMS.

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4. As the run progresses, the InterOp data files are filled in.
5. At the end of the run, the NovaSeq:
   - Copies the run data files to the InterOp folder.
   - Creates the `CopyComplete.txt` file in the run folder. This step indicates to the sequencing service that the run has completed and the data files are ready.
6. The sequencing service triggers the Read InterOp Metrics automation. This automation reads the files, records the parsed metrics into Clarity LIMS, and finally completes the step in Clarity LIMS.

Parse Run Parameters and Generate Link to Run Folder Automation

Automatically triggered on entry to the Record Details screen, this automation does the following actions:

- Parses the `RunParameters.xml` file attached on the Step Setup screen.
- Populates the master and global step fields with extracted values.
- If there is an instrument in Clarity LIMS that matches the instrument name in the file (InstrumentName field), the script associates this instrument with the run.
- Generates and attaches a link to the run folder using the run output folder parsed from `RunParameters.xml`.

The following rules and constraints are present:

- If there is no InstrumentName value in the file, the script continues, but it logs a warning.
- If there is an InstrumentName value in the file and multiple instruments with the same name exist in Clarity LIMS, the script fails and logs an error.
- If the `RunParameters.xml` file is not attached to the step during step setup, the script fails.
- Every `RunParameters.xml` field that has an equivalent step UDF/custom field in Clarity LIMS is required—except for InstrumentName. If a field is not present in the `RunParameters.xml` file, Clarity LIMS logs an error to this effect.

Master Step Fields

There are 23 fields defined on the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.2) step. The fields are shown in the Clarity LIMS Configuration area (Custom Fields > Master Step Fields tab).

The following table shows how the parsed `RunParameters.xml` fields map to step fields configured on the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.2) step in Clarity LIMS.

Table 274 Fields Parsed by parse_novaseq_run_parameters script

RunParameters.xml Field	Field Name
FlowCellSerialBarcode	Flow Cell ID

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RunParameters.xml Field	Field Name
FlowCellPartNumber	Flow Cell Part Number
FlowCellLotNumber	Flow Cell Lot Number
FlowCellExpirationdate	Flow Cell Expiration Date
FlowCellMode	Flow Cell Mode
RunId	Run ID
ClusterSerialBarcode	PE Serial Barcode
ClusterPartNumber	PE Part Number
ClusterLotNumber	PE Lot Number
ClusterExpirationdate	PE Expiration Date
ClusterCycleKit	PE Cycle Kit
SbsSerialBarcode	SBS Serial Barcode
SbsPartNumber	SBS Part Number
SbsLotNumber	SBS Lot Number
SbsExpirationdate	SBS Expiration Date
SbsCycleKit	SBS Cycle Kit
BufferSerialBarcode	Buffer Serial Barcode
BufferPartNumber	Buffer Part Number
BufferLotNumber	Buffer Lot Number
BufferExpirationdate	Buffer Expiration
OutputRunFolder	Output Folder
WorkflowType	Loading Workflow Type

Read InterOp Metrics Automation

Automatically triggered when the sequencing service detects the `CopyComplete.txt` file has been created, this automation parses the InterOp data generated by the run.

- Read summary metrics are recorded on the library pool. These values are aggregated across all lanes. Some values—for example Yield PF (Gb)—are summed, while others are averaged.
- Per lane metrics are recorded on the measurement outputs.

The `read_interop_metrics` script loads data from the InterOp files and records summary metrics into derived sample UDFs/custom fields in Clarity LIMS. If any field is not configured or cannot be written to, the script fails.

Global Fields

The global fields that are configured to support the parsing of key metrics back into Clarity LIMS are as follows.

- Yield PF (Gb) R1
- Yield PF (Gb) R2
- Reads PF (M) R1
- Reads PF (M) R2
- Cluster Density (K/mm^2) R1
- Cluster Density (K/mm^2) R2
- %PF R1
- %PF R2
- % Bases >=Q30 R1
- % Bases >=Q30 R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- % Aligned R1
- % Aligned R
- % Error Rate R1
- % Error Rate R2

Set Next Steps Automation

Automatically triggered on exit of the Record Details screen, the `nextStep = ::ADVANCE::` automation sets the next step to ADVANCE and the samples complete the protocol.

Update Lane Number Automation

Automatically triggered on exit of the Step Setup screen, this automation updates the lane number so that Read InterOp Metrics automation script can populate lane metric to the correct lane.



Do not disable Update Lane Number automation as it causes run metric parsing to work improperly.

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Components Installed

The following sections describe the various components that are installed by default as part of this integration. These components include files, database properties, reagent categories/label groups, reagent kits, and containers. Information on installed workflows, protocols, steps, and automation points is provided in the previous *Workflows, Protocols, and Steps* section.

The Illumina NovaSeq 6000 Integration v2.4 is distributed as two RPM packages:

- BaseSpaceLIMS-novaseq-extensions
- BaseSpaceLIMS-novaseq-sequencing-service

The RPM packages install the components listed in the following table.

The NovaSeq 6000 Integration requires installation of NGS Extensions Package v5.21 or later.

- If the NGS Extensions Package is not already installed, or if a version earlier than v5.21 is installed, the latest version is installed by default with the NovaSeq integration. See Release Notes for details.
- If NGS Extensions Package v5.21 is already installed, upgrade is not forced or required.
- Secret Util Package is required for the NGS Extensions Package v5.21.
- If the Secret Util Package is not already installed, then Secret Util package is installed along with the installation of NGS Extension Package v5.21.
- If the Secret Util Package is already installed, upgrade is not forced or required.

Table 275 IlluminaNovaSeq 6000 Integration RPM Components

Files Installed	Location and Description
configure_extensions_novaseq.sh	/opt/gls/clarity/config/ Script that imports Clarity LIMS configuration into the application database and updates property values.
novaseq-extensions.jar	/opt/gls/clarity/extensions/novaseq Jar file containing API-based Clarity LIMS extensions used throughout the protocols.
novaseq-sequencing.jar	/opt/gls/clarity/extensions/novaseq/SequencingService Sequencing service jar file that captures the run results.
novaseq-remote-extensions.jar	/opt/gls/clarity/extensions/novaseq Jar file containing scripts that require access to the run folders on the NAS.
InterOp libraries	/opt/gls/clarity/extensions/novaseq/lib Illumina shared library for parsing InterOp data files.

Files Installed	Location and Description
• NovaSeq_Standard_Bulk_Pool1.csv • NovaSeq_Standard_Bulk_Pool2.csv • NovaSeq_Standard_Bulk_Pool3.csv • NovaSeq_Xp_Bulk_Pool.csv • NovaSeq_Xp_Bulk_Pool2.csv • NovaSeq_Xp_Working_Pool.csv • NovaSeq_Xp_Working_Pool2.csv	/opt/gls/clarity/extensions/novaseq/Templates Template files used for file generation throughout the protocols.

Database Properties

The following table lists the database properties installed with the Illumina NovaSeq 6000 Integration Package.

Table 276 Database Properties Installed

Property	Description
novaseq.runSetupFolder	The directory where the run recipe files are written.
novaseq.sampleSheetPathPrefixSearch.SUFFIX	The directory where the instrument control software or analysis software looks for sample sheets, typically a Windows path to a network drive.
novaseq.sampleSheetPathPrefixReplace.SUFFIX	The directory where the sample sheets are written.
novaseq.sampleSheetPathPrefixSearchReplaceSuffixes*	Search and replace entries for transforming Windows to Linux network paths. This property must be set to the single SUFFIX used in the two previous properties.
novaseq.seqservice.netPathPrefixSearch.SUFFIX	The search prefix used to map the OutputRunFolder field in `RunParameters.xml` to the corresponding Linux directory.

Property	Description
novaseq.seqservice.netPathPrefixReplace.SUFFIX	The directory where the sequencing service looks for run folders.
novaseq.seqservice.netPathPrefixSearchReplaceSuffixes*	Search and replace entries for transforming Windows to Linux network paths. This property must be set to the single SUFFIX used in the two properties above.

*This version of the integration only supports a single active search-replace pair of each type:sampleSheetPathPrefixSearchReplaceSuffixes and netPathPrefixSearchReplaceSuffixesmust each contain a single number, rather than a comma-separated list of numbers.

Label Groups, Reagent Kits, and Containers

The NovaSeq 6000 Integration v2.4 installs the following label groups, reagent kits, and container types.

Label Groups

- TruSeq HT Adapters v2 (D7-D5)

Reagent Kits

- DPX1
- DPX2
- DPX3
- Resuspension Buffer (RSB)
- NaOH
- Tris HCl

Container Types

- Library Tube

Instrument Integration

The following are instructions for configuring the NovaSeq instrument for the Clarity LIMS integration:

1. Launch NovaSeq Control Software.
2. On the home screen, select the menu (top left) and select Settings.
3. Select File-Based and enter the network folder location that NovaSeq Control Software retrieves the JSON recipe from.
4. Next enter the network output folder location.
5. Select Save.

For more information, refer to the *NovaSeq Sequencing System Guide*.

Routing Script Requirements

The requirements for the routing script functionality are as follows.

- On the steps that use the routing script—Define Run Format (NovaSeq 6000 v2.2) and Dilute and Denature (NovaSeq 6000 v2.2)—the Next Step for all samples must be set to Remove from workflow. This value is set by a script. Do not change this value in the Assign Next Steps screen.
- In the protocol configuration screen, the following setting is required and must not be changed.
    - In the Next Steps section for the last step in the protocol, the method of assigning the next step must be set to Automatic.

Sample Sheet and Run Recipe File Generation

Sample sheet and run recipe file generation occurs on the step before the run—Dilute and Denature (NovaSeq 6000 v2.2) in the NovaSeq Standard protocol or Load to Flowcell (NovaSeq 6000 v2.2) in the NovaSeq Xp protocol. This step is where samples are placed on the library tube or flow cell that is loaded in the NovaSeq instrument.

In the default configuration, the Generate Sample Sheet and Run Recipe automation generates the following:

- One CSV format sample sheet file for use bcl2fastq v2.20 downstream analysis
- One JSON format run recipe file

The Prepare Files for NovaSeq automation places the generated files on the NAS where the instrument uses them to start the run.

How It Works

- The Prepare Files for NovaSeq automation command line invokes the `copy_attachments_to_network_folder` script `in novaseq-remote-extensions.jar`. The script can be used on a remote AI node/automation worker, in case the Clarity LIMS server does not have direct access to the NAS.
- The output folder for the run recipe file is found in the `novaseq.runSetupFolder` property.
- The output folder for the sample sheet is determined by using the `novaseq.sampleSheetPathSearchReplaceSuffixes` property. This property must contain exactly one suffix (not a comma-separated list) to derive a full property name based on `novaseq.sampleSheetPathPrefixReplace`.

Sample Sheet Contents

The contents of the sample sheet are controlled by the following fields configured on the Dilute and Denature (NovaSeq 6000 v2.2) and Load to Flowcell (NovaSeq 6000 v2.2) steps:

- Experiment Name
- Read 1 Cycles
- Read 2 Cycles
- Workflow
- Reverse Complement Workflow
- Read1UMILength
- Read2UMILength
- Read1StartFromCycle
- Read2StartFromCycle

Refer to the *Bcl2fastq2 Sample Sheet Generation* section of the *Illumina Instrument Sample Sheets (NGS v5.17 & later)* on page 2542 document for detailed information on the following:

- Sample sheet generation script parameters and usage
- Sample sheet data and configuration options
- Enabling unique FASTQ file names per sequencing run

Run Recipe Contents

The contents of the run recipe are controlled by the following fields. These fields are configured on the Dilute and Denature (NovaSeq 6000 v2.2) and Load to Flowcell (NovaSeq 6000 v2.2) steps.

- Experiment Name
- Run Mode
- Workflow Type
- Sample Loading Type (populated based on Workflow field)
- Paired End
- Read 1
- Read 2
- Index Read 1
- Index Read 2
- Sample Sheet Path
- BaseSpace Configuration

- Use Custom Read 1 Primer
- Use Custom Read 2 Primer
- Use Custom Index Read 1 Primer

The following fields are also included in the run recipe:

- Library Tube Barcode (librarytube_ID in JSON file)
  - NovaSeq Standard workflow—Library tube barcode is derived from the container name entered or scanned on the Placement screen.
  - NovaSeq Xp workflow—Library tube barcode is derived from the Library Tube Barcode field in the Load To Flowcell (NovaSeq 6000 v2.2) step.
- Flowcell Barcode (flowcell_ID in JSON file)
  - NovaSeq Standard workflow—Library tube barcode is not output to JSON recipe file.
  - NovaSeq Xp workflow—Flow cell barcode is derived from the container name entered or scanned on the Placement screen.
- Rehyb—preset to False.
- Output Folder (output_folder in JSON file)—Derived from the system property novaseq.seqservice.netPathPrefixSearch.
- Attachment—Derived from the system property novaseq.sampleSheetPathPrefixSearch with the name of the sample sheet added to the end of the path.
- Use BaseSpace (use_basespace in JSON file)—Set to False if the BaseSpace Sequence Hub Configuration step field value is Not Used, and true otherwise.

Rules and Constraints

- The sample sheet and run recipe files are mandatory inputs for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.2) step. For the automated run to start successfully, select the Generate Sample Sheet and Run Recipe button to generate these files.
- The output folders must exist on the system where the script runs (can be a remote AI node/automation worker).
- The output folders must be writable by the glsai user.

Container Types

The integration supports the following container types:

- Library tube with barcode provided in the following format:
  - [A-Za-z]{2}[0-9]{7}-[A-Za-z]{3}
  - Example—NV1234567-LIB
- Flow cell with barcode provided in one of the following formats:

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- For SP and S1—[A-Za-z0-9]{5}DRX[XY2357]
- For S2—[A-Za-z0-9]{5}DMX[XY2357]
- For S4—[A-Za-z0-9]{5}DSX[XY2357]
- Example S2 flow cell barcode—H1234DMXX

Rules and Constraints

- The workflow configuration contains several validation checks. To make sure that the calculations work properly, it is important that you do not disable any of this validation logic. The validation checks the following information:
  - Which samples, and how many, can enter each step together.
  - Which samples, and how many, can be pooled together.
- Reagent labels (indexes) must be unique.
- The library tube ID must be unique. There should not be multiple library tube containers in the system with the same name.
- Only controls are permitted as unindexed samples. All other unindexed samples and pools are not permitted.
- For sample sheet generation constraints, refer to Bcl2fastq2 Sample Sheet Generation.
- Do not manually start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.2) step. This step is fully automated. If the samples have been manually started, the sequencing service does not update them correctly.
- The output folder must be readable by the Clarity LIMS glsjboss account on the server. For details, see the NovaSeq 6000 Integration v2.4 Installation Guide.

NovaSeq 6000 Integration v2.4 Validation and Troubleshooting

This guide explains how to validate the installation of the Illumina NovaSeq 6000 Integration Package v2.4. The validation process involves the following actions:

- Running samples through the NovaSeq Validation Library Prep (NovaSeq 6000 v2.2) workflow. The workflow contains a single-step protocol that models the library prep required to produce normalized libraries. At the end of the step, the normalized libraries are automatically advanced to the NovaSeq 6000 v2.2 workflow.
- Running normalized libraries through the NovaSeq 6000 v2.2 workflow. This process validates the following details:
  - Successful routing of samples from the Run Format (NovaSeq 6000 v2.2) step to the NovaSeq Standard (NovaSeq 6000 v2.2) or NovaSeq Xp (NovaSeq 6000 v2.2) step.
  - Automated generation of a sample sheet for use with bcl2fastq2 v2.20 analysis software.

- Automated generation of a run recipe file (JSON file) with the library tube or flow cell barcode as the name (eg, NV1234567-LIB.json or H1234DRXX.json). This file is automatically uploaded to the sequencing instrument and used to set up and initiate the run.
- Automated tracking of the NovaSeq sequencing run and parsing of run statistics (per run per lane) into the LIMS.

Before executing the validation steps, make sure that you have installed the Illumina NovaSeq 6000 Integration Package v2.4 and have imported the default Clarity LIMS configuration.

Activate Workflow, Create Project, Add and Assign Samples

The following steps set up Clarity LIMS in preparation for running samples through the NovaSeq Validation Library Prep (NovaSeq 6000 v2.2) and NovaSeq 6000 v2.2 workflows.

1. In the Clarity LIMS Cconfiguration area, activate the NovaSeq Validation Library Prep (NovaSeq 6000 v2.2) and NovaSeq 6000 v2.2 workflows.
2. On the Projects and Samples screen, create a project and add samples to it.
3. Assign the samples to the NovaSeq Validation Library Prep (NovaSeq 6000 v2.2) workflow.

Library Prep Protocol: NovaSeq Validation Library Prep (NovaSeq 6000 v2.2)

This single-step protocol models the library prep required to produce normalized libraries that are ready for the NovaSeq 6000 v2.2 workflow.

Run NovaSeq Validation Library Prep (NovaSeq 6000 v2.2)

1. In Lab View, locate the NovaSeq Validation Library Prep (NovaSeq 6000 v2.2) protocol. The samples are queued for the NovaSeq Validation Library Prep (NovaSeq 6000 v2.2) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Placement screen, scan or enter the barcode of the destination container into the Container field of the Placed Samples area.
   a. Select the samples from the Samples to be Placed area on the left, then drag them over to the container wells on the right.
   b. Select Add Labels.
5. On the Add Labels screen, select labels from the Labels to Add list and drag them to the container on the right to assign them to placed samples.
   The TruSeq HT Adapters v2 (D7-D5) label group is the only label group configured in the out-of-the-box integration and is the default in this step.
   a. Select Record Details.
6. On the Record Details screen in the Sample Details table, enter values for Normalized Molarity. Type values individually or use the copy/paste functionality as follows.

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a. Select the Copy button to copy the sample information from Clarity LIMS to the clipboard.
b. Open a blank Excel document and paste the contents of the clipboard into the spreadsheet.
c. In the spreadsheet, enter the Normalized Molarity values. Select the entire spreadsheet, including the headers (Ctrl + A), and copy (Ctrl + C) the information to the clipboard.
d. In Clarity LIMS, select the Paste button. Paste the contents of the clipboard (Ctrl + V) into the Update Table window, then select Update to paste into the Sample Details table.

The Sequencer Type field indicates the instrument to be used for the sequencing run. In this integration, the value of this field is set to NovaSeq automatically.

7. Select Next Steps.
   This selection triggers the Next Steps automation, which sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step successfully.
8. On the Assign Next Steps screen in the Samples table, the Next Step for all samples is prepopulated with Remove from workflow.

   Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.

9. Select Finish Step.
   On exit from the step, the Routing Script automation is triggered. This automation assigns samples to the first step of the NovaSeq 6000 v2.2 workflow—Define Run Format (NovaSeq 6000 v2.2). This step is the only one in Protocol 1: Run Format (NovaSeq 6000 v2.2).

Protocol 1: Run Format (NovaSeq 6000 v2.2)

This protocol includes the Define Run Format (NovaSeq 6000 v2.2) step. The step allows for the assignment of per sample values for Loading Workflow Type, Normalized Molarity, Flowcell Type, and Final Loading Concentration (pM). At the end of the step, samples are routed to the NovaSeq Standard or NovaSeq Xp protocol, according to the selected Loading Workflow Type.

Run Define Run Format (NovaSeq 6000 v2.2) Step

1. In Lab View, locate the Run Format (NovaSeq 6000 v2.2) protocol. The samples are queued for the Define Run Format (NovaSeq 6000 v2.2) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Record Details screen, specify the Sample Details.
   - Loading Workflow Type—Select NovaSeq Standard or NovaSeq Xp from the drop-down list.
   - Normalized Molarity—Enter values or use values copied from the previous step.
5. Populate the following fields:

Values can vary across samples.

- Flowcell Type—Select SP, S1, S2, or S4.
- Final Loading Concentration (pM)—Select from the two preset options—225 (for PCR-free workflows) or 400 (for Nano workflows)—or enter a different value.

6. Select Next Steps to trigger the Next Steps automation, which does the following actions:
   - Sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step successfully.
   - Calculates the Minimum Molarity.
   - Checks Normalized Molarity value. For samples with no Normalized Molarity value (eg, an empty value, not including 0), the automation generates an error message stating that the field cannot be empty.
   - Compares the Normalized Molarity value of each sample with the Minimum Molarity value.
7. On the Assign Next Steps screen, review the Sample Details table and make sure that the Next Step for all samples is prepopulated with Remove from workflow.
   Next Step must be set to Remove from workflow, regardless of the Loading Workflow Type.

   If the Next Step value is not set to Remove from workflow, the routing script automation cannot advance to the next step.

8. Review the Warning field entries for the samples. If the field indicates that the Normalized Molarity is too low, continue with one of the following options:
   - Return to the Record Details screen and adjust the Normalized Molarity value so that it equals or exceeds the Minimum Molarity value. Set the Loading Workflow Type to NovaSeq Standard or NovaSeq Xp, as applicable.
   - Alternatively, complete the protocol without correcting the Normalized Molarity value. In this case, the samples in question are removed from the Clarity LIMS workflow.
9. Select Finish Step.
   The Routing Script automation is triggered if:
   - Samples with a Loading Workflow Type of Remove from workflow (ie, where Normalized Molarity value is lower than the Minimum Molarity) are removed from the Clarity LIMS workflow.
   - Samples with a Loading Workflow Type of NovaSeq Standard are routed to the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.2) step. This step is the first of two steps in Protocol 2: NovaSeq Standard (NovaSeq 6000 v2.2).
   - Samples with a Loading Workflow Type of NovaSeq Xp are routed to the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.2) step. This step is the first of three steps in Protocol 3: NovaSeq Xp (NovaSeq 6000 v2.2).

Protocol 2: NovaSeq Standard (NovaSeq 6000 v2.2)

In this protocol, samples are pooled and added to the library tube in preparation for the NovaSeq run. The protocol contains two steps:

1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.2)
2. Dilute and Denature (NovaSeq 6000 v2.2)

Step 1: Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.2)

1. In Lab View, locate the NovaSeq Standard (NovaSeq 6000 v2.2) protocol. The samples are queued for the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.2) step.
2. On the Queue screen, add samples of the same flow cell type to the Ice Bucket and select Begin Work.
3. On the Pooling screen, create a pool by dragging samples into the Pool Creator. Only one pool must be created.
   a. Type a name for the pool or accept the default name (Pool #1).
   b. Select Record Details.
4. On exit from the Pooling screen, the Validate Inputs Flowcell Type and Single Pool is triggered. The automation checks the following information:
   - All samples in the pool have been assigned the same Flowcell Type.
   - Only one pool has been created.
5. On the Record Details screen in the Step Details area, complete the fields as follows.
   a. Enter the value for the Number of Flowcells to Sequence field.
   This value is used in volume calculations to make sure that the volumes are sufficient for the number of times the pool is sequenced.
   b. Enter the value for the Minimum Per Sample Volume (µl) field.
   The value in this field is used to calculate how much of each sample is included in the pool. The field is prepopulated with the configured default value (5 µl), but the value can be edited if necessary.
   c. If necessary, enter the value for the % PhiX (2.5 nM) Spike-In field.
   The value in this field is used to calculate the volume of PhiX v3 control to be included in the pool for the given percentage of spike-in. The field is optional and can be edited.

   Assuming the default Minimum Per Sample Volume (µl) value of 5, for a given batch:
   - If the smallest Per Sample Volume (µl) value is less than 5, the LIMS automatically assigns a value of 5 to the sample's Adjusted Per Sample Volume (µl) field.
   - Clarity LIMS then adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.
6. In the Sample Details table, select the pool icon to view details on the pool composition.
7. Select Calculate Volumes to trigger the Calculate Volumes automation, which performs volume calculations based on the selected Flowcell Type:
   - Calculates Bulk Pool Volume (µl) value.

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- Calculates the Per Sample Volume (µl) value to be added to the pool.
- Calculates PhiX Volume (µl) value if % PhiX (2.5 nM) Spike-In has value.
- Calculates the Total Sample Volume (µl) value.
- If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) value.
- Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
- Populates the Volume of Pool to Denature (µl), NaOH Volume (µl) and Tris-HCl Volume (µl) columns of the Sample Details table. (Values are set by a script and are not editable.)
- The automation also generates the Calculation File (*csv) and attaches it to the step. This file contains volume information about the pool and the individual samples it contains. Select the file to download it, then open it in Excel.

8. Select Next Steps to trigger the Set Next Step automation.
   This automation sets the next step for samples to ADVANCE, advancing them to the Dilute and Denature (NovaSeq 6000 v2.2) step in the protocol.
9. Select Finish Step.

At the end of this step, the pool of samples automatically advances to the Dilute and Denature (NovaSeq 6000 v2.2) step.

Step 2: Dilute and Denature (NovaSeq 6000 v2.2)

1. In Lab View, locate the NovaSeq Standard (NovaSeq 6000 v2.2) protocol. The pool of samples is queued for the Dilute and Denature (NovaSeq 6000 v2.2) step.
2. Add the samples to the Ice Bucket and select Begin Work.
   At the beginning of the step, the Validate Single Input automation is triggered. This automation checks that there is only one container input to the step.
3. On the Placement screen, drag the pool into the library tube in the Placed Samples area.
   a. Scan or type the barcode of the library tube into the Library Tube field.
   b. Select Record Details.
   On exit of the Placement screen, the Validate Library Tube Barcode automation checks that the library tube barcode conforms to the barcode mask [A-Za-z]{2}[0-9]{7}-[A-Za-z]{3}. If not, an error message displays.
4. On the Record Details screen, the Reagent Lot Tracking section tracks the NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. To add and activate reagent lots, complete the following steps:
   a. In a new browser tab, open an additional instance of Clarity LIMS.
   b. Navigate to the Consumables configuration screen.
   c. Select the NaOH reagent kit and select New Lot.

d. Enter the lot details and select Save.

e. Set the Status of Reagent Lot toggle switch to Active.

f. Repeat this process to add and activate Resuspension Buffer and Tris-HCl reagent lots.

g. Close this browser tab and return to your original working tab. Refresh the page.

5. In the Reagent Lot Tracking section, select from the active lots displayed in each drop-down list. The fields displayed in the Step Details section are used to generate the sample sheet and run recipe files. Some of these fields are autopopulated and some must be completed manually. See the following table for details.

Table 277 Fields Displayed on Record Details Screen of Denature and Dilute (NovaSeq 6000 v2.2) Step

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. No spaces.
BaseSpace Sequence Hub Configuration	Select from preset options: Not Used, Run Monitoring Only, or Run Monitoring and Storage.
Workflow	Automatically populated with preset— GenerateFASTQ.
Workflow Type	Select from preset options: No Index, Single Index, or Dual Index.
Index Read 1	Select from preset options: 0, 6, or 8—or type a value between 0 and 20.
Index Read 2	Select from preset options: 0, 6, or 8—or type a value between 0 and 20.
Paired End	Select from preset options: True or False.
Read 1 Cycles	Select from preset options: 251, 151, 101, or 51—or type a value between 1 and 251.
Read 2 Cycles	Select from preset options: 251, 151, 101, or 51—or type a value between 1 and 251.
Use Custom Read 1 Primer	Select if applicable. LIMS v5.0.5 and later: See the Configuration Update documentation.
Use Custom Read 2 Primer	Select if applicable. LIMS v5.0.5 and later: See the Configuration Update documentation.
Use Custom Index Read 1 Primer	Select if applicable. LIMS v5.0.5 and later: See the Configuration Update documentation.

Field	Value
Reverse Complement Workflow	Select Yes (by default) for v1.5 reagents in NovaSeq Control Software v1.7 and onwards. Select No for v1 reagents.
UMI - Read 1 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 1. Leave blank if not applicable.
UMI - Read 2 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 2. Leave blank if not applicable.
UMI - Read 1 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.
UMI - Read 2 Start From Cycle	Enter the cycle number that Read 2 starts from. Leave blank if not applicable.
Sample Sheet Path	Automatically populated after the sample sheet has been generated.

6. On the Record Details screen, select Generate Sample Sheet and Run Recipe. This selection triggers the automation script: The sample sheet and JSON files are generated and attached to the placeholders in the Files area of the Record Details screen.
   The Sample Sheet Path field is populated with the path to the sample sheet file.
7. Select Next Steps. This selection triggers the Prepare Files for NovaSeq automation, which does the following:
   - Copies the sample sheet and run recipe files to the location specified during installation. The NovaSeq instrument software uses these files to set up the run.
   - Sets the value of the next step to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step successfully.
8. On the Assign Next Steps screen, the Next Step for samples is prepopulated with Remove from workflow.
   Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.
9. Select Finish Step.
   On exit from the step, the Routing Script automation is triggered and samples are routed to Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.2).
   In Lab View, the pool of samples is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.2) step.



Do not add samples to the Ice Bucket or start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.2) step. The integration does this action automatically.

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Protocol 3: NovaSeq Xp (NovaSeq 6000 v2.2)

In this protocol, samples are pooled and added to lanes on the NovaSeq flow cell. The flow cell type is determined by the option selected in the Define Run Format (NovaSeq 6000 v2.2) step.

The protocol contains three steps:

1. Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.2)
2. Dilute, Denature and ExAmp (NovaSeq 6000 v2.2)
3. Load to Flowcell (NovaSeq 6000 v2.2)

Step 1: Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.2)

1. In Lab View, locate the NovaSeq Standard (NovaSeq 6000 v2.2) protocol. The samples are queued for the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.2) step.
2. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucket and select Begin Work.
3. On the Pooling screen, create a pool by dragging samples into the Pool Creator. Only one pool must be created.
   a. Type a name for the pool or accept the default name Pool #1.
   b. Select Record Details.
   On exit from the Pooling screen, the Validate Inputs Flowcell Type and Single Pool is triggered. The automation checks the following details:
   - All samples in the pool have been assigned the same Flowcell Type.
   - Only one pool has been created.
4. On the Record Details screen in the Step Details area, complete the fields as follows.
   a. Enter the value for the Number of Lanes to Sequence field.
   This value is used in volume calculations to make sure that the volumes are sufficient for the number of times the pool is sequenced.
   b. Enter the value for the Minimum Per Sample Volume (µl) field.
   The value in this field is used to calculate how much of each sample is included in the pool. The field is prepopulated with the configured default value (5 µl), but the value can be edited if necessary.
   c. If necessary, enter the value for the % PhiX (0.25 nM) Spike-In field.
   The value in this field is used to calculate the volume of PhiX v3 control to be included in the pool for the given percentage of spike-in. The field is optional and can be edited.

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Assuming the default Minimum Per Sample Volume (µl) value of 5, for a given batch:

- If the smallest Per Sample Volume (µl) value is less than 5, the LIMS automatically assigns a value of 5 to the sample's Adjusted Per Sample Volume (µl) field.
- Clarity LIMS then adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

5. In the Sample Details table, select the pool icon to view details on the pool composition.
6. Select Calculate Volumes to trigger the Calculate Volumes automation, which performs volume calculations based on the selected Flowcell Type:
   - Calculates Bulk Pool Volume (µl) value.
   - Calculates the Per Sample Volume (µl) value to be added to the pool.
   - Calculates PhiX Volume (µl) value if % PhiX (2.5 nM) Spike-In has value.
   - Calculates the Total Sample Volume (µl) value.
   - If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) value.
   - Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
   - The automation also generates the Calculation File (*csv) and attaches it to the step. This file contains volume information about the samples and RSB buffer to add to the pool. Select the file to download it, then open it in Excel.
7. Select Next Steps to trigger the Set Next Step automation.
   This automation sets the next step for samples to ADVANCE, advancing them to the Dilute, Denature and ExAmp (NovaSeq 6000 v2.2) step in the protocol.
8. Select Finish Step.

At the end of this step, the pool of samples automatically advances to the Dilute, Denature and ExAmp (NovaSeq 6000 v2.2) step.

Step 2: Dilute, Denature and ExAmp (NovaSeq 6000 v2.2)

1. In Lab View, locate the NovaSeq Xp (NovaSeq 6000 v2.2) protocol. The pool of samples is queued for the Dilute, Denature and ExAmp (NovaSeq 6000 v2.2) step. Add the pool to the Ice Bucket.
2. On the Ice Bucket screen, set the number of derivatives to create (they are placed into the flow cell lanes) and select Begin Work.
   The Validate Inputs Flowcell Type automation checks that there is only one container input to the step.
3. On entry to the Record Details screen, the Calculate Volumes automation is triggered.
   This automation sets the following values based on the selected Flowcell Type:
   - NaOH Volume (µl)

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- Tris-HCl Volume (µl)
- DPX1 Volume (µl)
- DPX2 Volume (µl)
- DPX3 Volume (µl)
- Mastermix per Lane (µl)
- The automation also generates the Calculation File (*csv) and attaches it to the step. This file contains information about the DPX Mastermix volume and the volume of Mastermix, NaOH, and Tris-HCl to add per working pool.

4. On the Record Details screen, the Reagent Lot Tracking section tracks the DPX1, DPX2, DPX3, NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. To add and activate reagent lots, complete the following steps:
   a. In a new browser tab, open an additional instance of Clarity LIMS.
   b. Navigate to the Consumables configuration screen.
   c. Select one of the reagent kits listed and select New Lot.
   d. Enter the lot details and select Save.
   e. Set the Status of Reagent Lot toggle switch to Active.
   f. Repeat this process to add and activate the remaining reagent lots.
   g. Close this browser tab and return to your original working tab. Refresh the page.
5. On the Record Details screen in the Reagent Lot Tracking section, select from the active lots displayed in each drop-down list.
   In the Step Details area, the DPX1, DPX2, and DPX3 reagent volume values are already populated. These values are set by a script and are not editable while running the step.
6. In the Sample Details table, select the pool icon to view details on the working pool composition.

   The Bp Aliquot, Mastermix per lane, NaOH, and Tris-HCl volume values for each working pool are already populated. These values are set by a script and are not editable while running the step.

   The working pool number is appended to the bulk pool name. This feature allows you to identify which working pools are derived from the same bulk pool quickly.

7. Select Next Steps.
   On the Assign Next Steps screen, the next step is already set to Load to Flowcell (NovaSeq 6000 v2.2).
8. Select Finish Step.

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Step 3: Run Load to Flowcell (NovaSeq 6000 v2.2)

1. On the Ice Bucket screen in the Container Options panel, select the appropriate flow cell type from the Destination Container drop-down list and select Begin Work.
2. On the Placement screen, drag the pools from the left of the screen over into the Placed Samples area on the right.
   a. Scan or type the barcode of the flow cell into the Flow Cell field.
   b. Select Record Details.
   On exit of the Placement screen, the Validate Flowcell Barcode automation validates the container barcode.
3. The fields displayed in the Step Details section are used to generate the sample sheet and run recipe files. Some of these fields are autopopulated and some must be completed manually. See the following table for details.

Table 278 Fields Displayed on Record Details Screen of Load to Flowcell (NovaSeq 6000 v2.2) Step

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. No spaces.
BaseSpace Sequence Hub Configuration	Select from preset options: Not Used, Run Monitoring Only, or Run Monitoring and Storage.
Workflow	Automatically populated with preset - GenerateFASTQ.
Workflow Type	Select from preset options: No Index, Single Index, and Dual Index.
Index Read 1	Select from preset options: 0, 6, or 8—or type a value between 0 and 20.
Index Read 2	Select from preset options: 0, 6, or 8—or type a value between 0 and 20.
Paired End	Select from preset options: True or False.
Read 1 Cycles	Select from preset options: 251, 151, 101, or 51—or type a value between 1 and 251.
Read 2 Cycles	Select from preset options: 251, 151, 101, or 51—or type a value between 1 and 251.
Use Custom Read 1 Primer	• Select if applicable.

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Field	Value
Use Custom Read 2 Primer	• Select, if applicable.
Use Custom Index Read 1 Primer	• Select, if applicable.
Reverse Complement Workflow	For v1.5 reagents in the NovaSeq Control Software (v1.7 and later), select Yes (which is the default). For v1 reagents, select No.
UMI - Read 1 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 1. Leave blank if not applicable.
UMI - Read 2 Length	Enter the length of the UMI in Read 2. Leave blank if not applicable.
UMI - Read 1 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.
UMI - Read 2 Start From Cycle	Enter the cycle number that Read 2 starts from. Leave blank if not applicable.
Sample Sheet Path	Automatically populated after the sample sheet has been generated.
Library Tube Barcode	Scan the library tube barcode.

4. Select Generate Sample Sheet and Run Recipe. This selection triggers the automation script, which:
   - Generates and attaches the sample sheet and JSON files to the placeholders in the Files area of the Record Details screen.
   - Populates the Sample Sheet Path field with the path to the sample sheet file.
5. Select Next Steps.
   This selection triggers the Prepare Files for NovaSeq automation, which copies the sample sheet and run recipe files to the location specified during installation. The NovaSeq instrument software uses these files to set up the run.
6. On the Assign Next Steps screen, make sure the Next Step for samples is prepopulated with Mark protocol as complete.
7. Select Finish Step.

At this point in the workflow, the user interaction ends. The flow cell is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.2) step.

Proceed to Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.2).

Do not add samples to the Ice Bucket or start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.2) step. The integration does this action automatically.

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Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.2)

This protocol contains a single fully automated step - AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.2).

The integration starts the step automatically and data from the run is parsed back into Clarity LIMS. No user interaction is required. However, you can open and review the various stages of the step in Clarity LIMS.

How the Integration Works

1. When initiating a run on the NovaSeq instrument, the NovaSeq Control Software (NVCS) looks for the sample sheet and run recipe (*.json file) on the shared network drive. The NVCS copies the sample sheet to the run directory.
   The NVCS uses the run recipe to start the sequencing run.
2. When the run starts, the instrument software creates a new run folder on the shared network drive and copies the following files into that folder:
   - `RunParameters.xml`
   - `RunInfo.xml`
   - LIMS/ <ContainerID>.json file (run recipe)
   - The Real-Time Analysis v3 (RTA3) software creates the InterOp folder.
3. The sequencing service detects the presence of the `RunParameters`, `RunInfo`, and JSON files and starts the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.2) step in Clarity LIMS.
4. As the run progresses, the InterOp data files are filled in.
5. At the end of the run, the NovaSeq creates the `CopyComplete.txt` file in the InterOp folder, which indicates to the sequencing service that the run has completed and the data files are ready.
6. The NovaSeq copies the run data files to the InterOp folder and the sequencing service triggers the Read InterOp Metrics automation. This automation reads the files, records the parsed metrics into Clarity LIMS, and completes the step in Clarity LIMS.

Parse Run Parameters and Generate Link to Run Folder Automation

The Parse Run Parameters and Generate Link to Run Folder automation is automatically triggered on entry to the Record Details screen. This automation parses the `RunParameters.xml` file attached on the Step Setup screen and populates the global/master step fields with extracted values. For details on this automation, including how the parsed fields map to the master step fields in Clarity LIMS, see *NovaSeq 6000 Integration v2.4 Configuration & User Interaction* on page 1085.

Read InterOp Metrics Automation

Automatically triggered when the sequencing service detects the `CopyComplete.txt` file has been created, this automation parses the InterOp data generated by the run.

The `read_interop_metrics` script records summary metrics into the following global fields in Clarity LIMS. The script fails if any field is not configured or cannot be written to.

- Yield PF (Gb) R1
- Yield PF (Gb) R2
- Reads PF (M) R1
- Reads PF (M) R2
- Cluster Density (K/mm^2) R1
- Cluster Density (K/mm^2) R2
- %PF R1
- %PF R2
- % Bases >=Q30 R1
- % Bases >=Q30 R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- % Aligned R1
- % Aligned R
- % Error Rate R1
- % Error Rate R2

For details, see the Configuration and User Interaction documentation.

Set Next Steps Automation

After the run metrics are parsed successfully, the Set Next Steps automation sets the next step for all samples to Mark protocol as complete. For details, see the Configuration and User Interaction documentation.

Troubleshooting

If an automation trigger does not appear to run its corresponding scripts, see the following sections in the Clarity LIMS API documentation:

- Troubleshooting Automated Informatics
- Troubleshooting Automation in the API

If an error occurs that does not provide direction on how to proceed, complete the following steps:

1. Confirm the version of the installed Illumina NovaSeq Integration Package by running the `rpm -qa | grep -i novaseq` command as follows.
   - For an on-premise deployment, run the command on Clarity LIMS server command line. This command retrieves the version of both RPMs installed.
   - For a cloud hosted deployment, run the command on the local server installation. This command retrieves the version of the remote extensions RPM installed.
2. If the error is related to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.2) step, there are two places to check for log file information:
   - If the step does not start, check the NovaSeqIntegrator.log log file written to:

```
/opt/gls/clarity/extensions/novaseq/SequencingService/NovaSeqIntegrator.log
```

     This log file is written to this path wherever the remote extensions RPM was installed.
   - If the step starts but does not complete, open the step in Clarity LIMS (it can be found via a search). On the Record Details screen, the log file is attached to a placeholder called Log File. If you are unable to reach the Record Details screen, or if the file does not contain an error, review the `NovaSeqIntegrator.log` described previously.
3. Contact the Illumina Support team, supplying the relevant information from the troubleshooting steps already performed.

NovaSeq 6000 Integration v2.4 Manual Upgrade

NovaSeq 6000 Integration Package v2.4 includes the preconfigured NovaSeq 6000 v2.2 workflow. The integration includes support for Clarity LIMS v6.0 and issue fixes. Refer to the Release Notes for details. Clarity LIMS v4.x is no longer supported in this integration package and later versions.

To keep the v2.1 workflow and upgrade to the v2.4 integration features, you can manually upgrade the configuration.

Before you manually upgrade, make sure the following items are installed:

- NGS Extension Package (v5.21 or later)
- Secret Utility v1.2

The NGS Extension Package v5.21 update is included in the NovaSeq 6000 Integration v2.4 upgrade. Secret Utility is also installed as part of NGS Extension Package v5.21.

Installation Steps

1. Install NGS v5.21 with Secret Utility v1.2.
2. Configure Secret Utility. Refer to the Secret Utility Configuration section that follows.
3. Install the BaseSpaceLIMS-novaseq-extensions v2.4.0 RPM.

When prompted, do not run the following script:
`/opt/gls/clarity/config/configure_extensions_novaseq.sh`

4. Install the BaseSpaceLIMS-novaseq-sequencing-service v2.4.0 RPM.
5. Manually upgrade your configuration using the instructions provided in the following Update NovaSeq Standard (NovaSeq 6000 v2.0) Protocol and Update NovaSeq Xp (NovaSeq 6000 v2.0) Protocol sections.

Configure Secret Utility

If Clarity LIMS Secret Utility is not installed or configured, use the following information to configure Secret Utility.

- Installation on a hosted system with Clarity LIMS (v5.4 or later)—The Clarity LIMS installation tooling configures this installation. No additional configuration is necessary.
- Installation on Clarity LIMS v5.3 (or other versions that are v5.1 and later)—The installation includes the following passwords:
  - Tenant DB password
  - Clarity DB password
  - FTP password
  - LDAP manager password (if LDAP is enabled)
  - LabLink DB password (for Clarity LIMS v5.3 LabLink only)
  - Reporting DB password
  - RabbitMQ password

For details on the Secret Utility configuration, see *Guide to Secret Management* in the Clarity LIMS Core Functionality documentation.

Update NovaSeq Standard (NovaSeq 6000 v2.0) Protocol

The manual integration upgrade starts with updates to the NovaSeq Standard (NovaSeq 6000 v2.0) Protocol.

Update the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0) step as follows.

1. In Clarity LIMS, under Configuration, select the Custom Fields tab.
2. Under the Master Step Fields tab, select the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0) master step.
3. Create a custom field named Calculate Volumes.
4. For Field Type, select Text.
5. In Additional Options, enter the following in the Default field:

```
step.::PhiX Volume (ul):: = !step.hasValue(::% PhiX (2.5nM) Spike-In::) ? 0 :
step.::% PhiX (2.5nM) Spike-In::; if (input.::Flowcell Type:: == ::SP::) {
step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 100
+ 30; step.::PhiX Volume (ul):: *= 0.8 }; if (input.::Flowcell Type:: == ::S1::)
{ step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: *
100 + 30; step.::PhiX Volume (ul):: *= 0.8 }; if (input.::Flowcell Type:: ==
::S2::) { step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to
Sequence:: * 150 + 30; step.::PhiX Volume (ul):: *= 1.1 }; if (input.::Flowcell
Type:: == ::S4::) { step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells
to Sequence:: * 310 + 30; step.::PhiX Volume (ul):: *= 2.1 }; if (step.::PhiX
Volume (ul):: == 0) { step.::PhiX Volume (ul):: = :::: }; input.::Per Sample
Volume (ul):: = (((input.::Final Loading Concentration (pM):: * 5 / 1000) /
input.::Normalized Molarity (nM)::) * step.::Bulk Pool Volume (ul)::) /
step.::Number of Samples in Pool::
```

6. For Field Options, select the following:
    - Required Field—Yes
    - Read Only—Yes
7. Select Save.
8. Modify the Calculate Volumes automation as follows.
    a. From Configuration, select the Automation tab.
    b. Under the Step Automation tab, select the Calculate Volumes automation that is enabled on the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0) master step.
    c. Update the default command line to the value provided in the drop-down section that follows.

Updated Calculate Volumes Command Line

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
-t true -h false -log {compoundOutputFileLuid1} \
script:evaluateDynamicExpression \
-exp 'step.::Number of Samples in Pool:: = step.::Number of Samples in Pool::
+ 1' \
script:evaluateDynamicExpression \
-exp '{udf:Calculate Volumes Script}' \
&& /opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/novaseq/novaseq-extensions.jar script:calculate_
adjusted_per_sample_volume -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
-t true -h false -log {compoundOutputFileLuid1} \
```


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```
script:evaluateDynamicExpression \
-exp 'step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: +
input.::Adjusted Per Sample Volume (ul)::' \
script:evaluateDynamicExpression \
-exp 'if (step.::Total Sample Volume (ul):: >= step.::Bulk Pool Volume
(ul)::) {output.::RSB Volume (ul):: = 0} else {output.::RSB Volume (ul):: =
step.::Bulk Pool Volume (ul):: - step.::Total Sample Volume (ul)::} ;
output.::Flowcell Type:: = input.::Flowcell Type:: ; output.::Loading
Workflow Type:: = input.::Loading Workflow Type:: ; if ( input.::Flowcell
Type:: == ::SP:: ) { output.::Volume of Pool to Denature (ul):: = 100 ;
output.::NaOH Volume (ul):: = 25 ; output.::Tris-HCl Volume (ul):: = 25 } ;
if ( input.::Flowcell Type:: == ::S1:: ) { output.::Volume of Pool to
Denature (ul):: = 100 ; output.::NaOH Volume (ul):: = 25 ; output.::Tris-HCl
Volume (ul):: = 25 } ; if ( input.::Flowcell Type:: == ::S2:: )
{output.::Volume of Pool to Denature (ul):: = 150 ; output.::NaOH Volume
(ul):: = 37.50 ; output.::Tris-HCl Volume (ul):: = 37.50 } ; ; if (
input.::Flowcell Type:: == ::S4:: ) { output.::Volume of Pool to Denature
(ul):: = 310 ; output.::NaOH Volume (ul):: = 77.50 ; output.::Tris-HCl Volume
(ul):: = 77.50 }' \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {processURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Standard_Bulk_
Pool1.csv \
-o 1.csv \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Standard_Bulk_
Pool2.csv \
-o 2.csv \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Standard_Bulk_
Pool3.csv \
-o 3.csv \
&& cat 1.csv 2.csv 3.csv > {compoundOutputFileLuid0}.csv \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
-t true -log {compoundOutputFileLuid1} \
script:evaluateDynamicExpression \
-h false \
```


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```
-exp 'step.::Number of Samples in Pool:: = step.::Number of Samples in Pool::
- 1; step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: -
input.::Adjusted Per Sample Volume (ul)::'"
```

You can also update the existing automation by replacing the second -exp section in the Command Line with `'{udf:Calculate Volumes Script}'`. Also, the first line of the following code is added to the last line of the original script:

The last line of the original script ends with `{compoundOutputFileLuid0}.csv > \`.

```
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
-t true -log {compoundOutputFileLuid1} \
script:evaluateDynamicExpression \
-h false \
-exp 'step.::Number of Samples in Pool:: = step.::Number of Samples in Pool::
- 1; step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: -
input.::Adjusted Per Sample Volume (ul)::'"
```

d. Select Save.

e. Make sure the start of the script contains `-exp '{udf:Calculate Volumes Script}' \` and that the bottom of the script matches the content of the Updated Calculate Volumes Script drop-down section.

Update the Generate Sample Sheet & Run Recipe Automation on the Dilute and Denature (NovaSeq 6000 v2.1) step as follows.

1. From Configuration, select the Automation tab.
2. Under the Step Automation tab, select the Generate Sample Sheet & Run Recipe automation that is enabled on the Dilute and Denature (NovaSeq 6000 v2.1) master step.
3. Update the command line to the default value provided in the drop-down section that follows.

Updated Generate Sample Sheet & Run Recipe Command Line

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
\
script:evaluateDynamicExpression \
-exp 'output.::Flowcell Type:: = input.::Flowcell Type::; step.::Run Mode:: =
input.::Flowcell Type::; output.::Loading Workflow Type:: = input.::Loading
Workflow Type::; if (!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) {
fail(::Experiment Name contains prohibited characters. Allowed characters
are: a-z, A-Z, 0-9, -, and _::) }; if (step.::Workflow Type:: == ::No
Index::) { if (step.::Index Read 1:: != 0 || step.::Index Read 2:: != 0) {
fail(::Index Read 1 and Index Read 2 must be 0 if No Index is selected.::) }
```


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```
} else if (step.::Workflow Type:: == ::Single Index::) { if (step.::Index
Read 1:: <= 0 || step.::Index Read 2:: != 0) { fail(::Index Read 1 must be
greater than 0 and Index Read 2 must be 0 if Single Index is selected.::) } }
else { if (step.::Index Read 1:: <= 0 || step.::Index Read 2:: <= 0) { fail
(::Index Read 1 and Index Read 2 must be greater than 0 if Dual Index is
selected.::) } }; if (step.::Paired End::.toBoolean()) { if (step.::Read 1
Cycles:: == 0 || step.::Read 2 Cycles:: == 0) { fail(::Read 1 Cycles and Read
2 Cycles must be greater than 0 if Paired End is True.::) } } else { if
(step.::Read 2 Cycles:: != 0) { fail(::Read 2 Cycles must be 0 if Paired End
is False.::) }; if (step.hasValue(::UMI - Read 2 Length::) || step.hasValue
(::UMI - Read 2 Start From Cycle::)) { fail(::UMI - Read 2 Length and UMI -
Read 2 Start From Cycle cannot be defined if Paired End is False.::) } }; if
(input.::Flowcell Type:: != ::SP:: && step.::Read 1 Cycles:: > 151) { fail
(::Read 1 Cycles must not be larger than 151 if it is not SPrime Flowcell.::)
}; if (input.::Flowcell Type:: != ::SP:: && step.::Read 2 Cycles:: > 151) {
fail(::Read 2 Cycles must not be larger than 151 if it is not SPrime
Flowcell.::) }; if (step.hasValue(::UMI - Read 1 Length::) && !step.hasValue
(::UMI - Read 1 Start From Cycle::)) { fail(::UMI - Read 1 Start From Cycle
must be greater than 0 if UMI - Read 1 Length is greater than 0.::) }; if
(!step.hasValue(::UMI - Read 1 Length::) && step.hasValue(::UMI - Read 1
Start From Cycle::)) { fail(::UMI - Read 1 Length must be greater than 0 if
UMI - Read 1 Start From Cycle is greater than 0.::) }; if (step.hasValue
(::UMI - Read 2 Length::) && !step.hasValue(::UMI - Read 2 Start From
Cycle::)) { fail(::UMI - Read 2 Start From Cycle must be greater than 0 if
UMI - Read 2 Length is greater than 0.::) }; if (!step.hasValue(::UMI - Read
2 Length::) && step.hasValue(::UMI - Read 2 Start From Cycle::)) { fail(::UMI
- Read 2 Length must be greater than 0 if UMI - Read 2 Start From Cycle is
greater than 0.::) }; if (!step.hasValue(::UMI - Read 1 Length::) &&
step.hasValue(::UMI - Read 2 Length::)) { fail(::UMI - Read 1 Length must be
greater than 0 if UMI - Read 2 Length is greater than 0.::) }; if
(!step.hasValue(::UMI - Read 1 Start From Cycle::) && step.hasValue(::UMI -
Read 2 Start From Cycle::)) { fail(::UMI - Read 1 Start From Cycle must be
greater than 0 if UMI - Read 2 Start From Cycle is greater than 0.::) };
nextStep = ::REMOVE::' \
-log {compoundOutputFileLuid2} \
-t true \
&& /opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/novaseq/novaseq-extensions.jar -u {username} -p
{password} -e {compoundOutputFileLuid2} \
script:generate_novaseq_sample_sheet \
-i {processURI:v2} \
```


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```
-c {compoundOutputFileLuid1} \
-r {udf:Reverse Complement Workflow} \
script:generate_novaseq_run_recipe \
-i {stepURI:v2} \
-c {compoundOutputFileLuid0}"
```

You can also update the existing automation by deleting the following from the first -exp script.

```
if (step.::Reverse Complement Workflow::.toBoolean()) { fail(::Reverse
Complement Workflow can only be enabled in a Paired End run.::) };
```

4. Select Save.

Update NovaSeq Xp (NovaSeq 6000 v2.0) Protocol

The manual integration upgrade continues with updates to the NovaSeq Xp (NovaSeq 6000 v2.0) protocol.

Update the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.0) step as follows.

1. From Configuration, select the Automation tab.
2. Under the Step Automation tab, select the Calculate Volumes automation that is enabled on the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.0) master step.
3. Update the command line to the default value provided in the drop-down section that follows.

Updated Calculate Volumes Command Line

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
-t true -h false -log {compoundOutputFileLuid1} \
script:evaluateDynamicExpression \
-exp 'step.::Number of Samples in Pool:: = step.::Number of Samples in Pool::
+ 1' \
script:evaluateDynamicExpression \
-exp 'step.::PhiX Volume (ul):: = !step.hasValue(::% PhiX (0.25nM) Spike-
In::) ? 0 : step.::% PhiX (0.25nM) Spike-In::; if (input.::Flowcell Type:: ==
::SP::) { step.::Bulk Pool Volume (ul):: = step.::Number of Lanes to
Sequence:: * 18 + 30; step.::PhiX Volume (ul):: *= 1.9 }; if
(input.::Flowcell Type:: == ::S1::) { step.::Bulk Pool Volume (ul):: =
step.::Number of Lanes to Sequence:: * 18 + 30; step.::PhiX Volume (ul):: *=
1.9 }; if (input.::Flowcell Type:: == ::S2::) { step.::Bulk Pool Volume
(ul):: = step.::Number of Lanes to Sequence:: * 22 + 30; step.::PhiX Volume
(ul):: *= 1.9 }; if (input.::Flowcell Type:: == ::S4::) { step.::Bulk Pool
Volume (ul):: = step.::Number of Lanes to Sequence:: * 30 + 30; step.::PhiX
Volume (ul):: *= 2.2 }; if (step.::PhiX Volume (ul):: == 0) { step.::PhiX
```


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```
Volume (ul):: = :::: }; input.::Per Sample Volume (ul):: = (((input.::Final
Loading Concentration (pM):: * 5 / 1000) / input.::Normalized Molarity
(nM)::) * step.::Bulk Pool Volume (ul)::) / step.::Number of Samples in
Pool::' \
&& /opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/novaseq/novaseq-extensions.jar script:calculate_
adjusted_per_sample_volume -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
-t true -h false -log {compoundOutputFileLuid1} \
script:evaluateDynamicExpression \
-exp 'step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: +
input.::Adjusted Per Sample Volume (ul)::' \
script:evaluateDynamicExpression \
-exp 'if (step.::Total Sample Volume (ul):: >= step.::Bulk Pool Volume
(ul)::) {output.::RSB Volume (ul):: = 0} else {output.::RSB Volume (ul):: =
step.::Bulk Pool Volume (ul):: - step.::Total Sample Volume (ul)::} ;
output.::Flowcell Type:: = input.::Flowcell Type:: ; output.::Loading
Workflow Type:: = input.::Loading Workflow Type::' \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {processURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Xp_Bulk_Pool.csv \
-o {compoundOutputFileLuid0}.csv \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Xp_Bulk_Pool2.csv \
-o append.csv \
&& cat append.csv >> {compoundOutputFileLuid0}.csv \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
-t true -log {compoundOutputFileLuid1} \
script:evaluateDynamicExpression \
-h false \
-exp 'step.::Number of Samples in Pool:: = step.::Number of Samples in Pool::
- 1; step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: -
input.::Adjusted Per Sample Volume (ul)::'"
```

You can also update the existing automation by appending the following to the end of the original script:

The last line of the original script ends with `{compoundOutputFileLuid0}.csv > \.`

```
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
-t true -log {compoundOutputFileLuid1} \
script:evaluateDynamicExpression \
-h false \
-exp 'step.::Number of Samples in Pool:: = step.::Number of Samples in Pool::
- 1; step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: -
input.::Adjusted Per Sample Volume (ul)::'"
```

4. Select Save.

Update the Generate Sample Sheet & Run Recipe Automation on the Load to Flowcell (NovaSeq 6000 v2.1) step as follows.

1. From Configuration, select the Automation tab.
2. Under the Step Automation tab, select the Generate Sample Sheet & Run Recipe automation that is enabled on the Load to Flowcell (NovaSeq 6000 v2.1) master step.
3. Update the command line to the default value provided in the drop-down section that follows.

Updated Generate Sample Sheet & Run Recipe Command Line

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-exp 'step.::Run Mode:: = input.::Flowcell Type::; if (!step.::Experiment
Name::.matches(::[a-zA-Z0-9-_]+::)) { fail(::Experiment Name contains prohibited
characters. Allowed characters are: a-z, A-Z, 0-9, -, and _::) }; if
(step.::Workflow Type:: == ::No Index::) { if (step.::Index Read 1:: != 0 ||
step.::Index Read 2:: != 0) { fail(::Index Read 1 and Index Read 2 must be 0 if
No Index is selected.::) } } else if (step.::Workflow Type:: == ::Single
Index::) { if (step.::Index Read 1:: <= 0 || step.::Index Read 2:: != 0) { fail
(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if Single
Index is selected.::) } } else { if (step.::Index Read 1:: <= 0 || step.::Index
Read 2:: <= 0) { fail(::Index Read 1 and Index Read 2 must be greater than 0 if
Dual Index is selected.::) } }; if (step.::Paired End::.toBoolean()) { if
(step.::Read 1 Cycles:: == 0 || step.::Read 2 Cycles:: == 0) { fail(::Read 1
Cycles and Read 2 Cycles must be greater than 0 if Paired End is True.::) } }
else { if (step.::Read 2 Cycles:: != 0) { fail(::Read 2 Cycles must be 0 if
Paired End is False.::) }; if (step.hasValue(::UMI - Read 2 Length::) ||
step.hasValue(::UMI - Read 2 Start From Cycle::)) { fail(::UMI - Read 2 Length
and UMI - Read 2 Start From Cycle cannot be defined if Paired End is False.::) }
}; if (step.hasValue(::UMI - Read 1 Length::) && !step.hasValue(::UMI - Read 1
```

```
Start From Cycle::)) { fail(::UMI - Read 1 Start From Cycle must be greater than
0 if UMI - Read 1 Length is greater than 0.::) }; if (!step.hasValue(::UMI -
Read 1 Length::) && step.hasValue(::UMI - Read 1 Start From Cycle::)) { fail
(::UMI - Read 1 Length must be greater than 0 if UMI - Read 1 Start From Cycle
is greater than 0.::) }; if (step.hasValue(::UMI - Read 2 Length::) &&
!step.hasValue(::UMI - Read 2 Start From Cycle::)) { fail(::UMI - Read 2 Start
From Cycle must be greater than 0 if UMI - Read 2 Length is greater than 0.::)
}; if (!step.hasValue(::UMI - Read 2 Length::) && step.hasValue(::UMI - Read 2
Start From Cycle::)) { fail(::UMI - Read 2 Length must be greater than 0 if UMI
- Read 2 Start From Cycle is greater than 0.::) }; if (!step.hasValue(::UMI -
Read 1 Length::) && step.hasValue(::UMI - Read 2 Length::)) { fail(::UMI - Read
1 Length must be greater than 0 if UMI - Read 2 Length is greater than 0.::) };
if (!step.hasValue(::UMI - Read 1 Start From Cycle::) && step.hasValue(::UMI -
Read 2 Start From Cycle::)) { fail(::UMI - Read 1 Start From Cycle must be
greater than 0 if UMI - Read 2 Start From Cycle is greater than 0.::) };
output.::Flowcell Type:: = input.::Flowcell Type::; if (input.::Flowcell Type::
!= ::SP:: && step.::Read 1 Cycles:: > 151) { fail(::Read 1 Cycles must not be
larger than 151 if it is not SPrime Flowcell.::) }; if (input.::Flowcell Type::
!= ::SP:: && step.::Read 2 Cycles:: > 151) { fail(::Read 2 Cycles must not be
larger than 151 if it is not SPrime Flowcell.::) }; nextStep = ::ADVANCE::' \
-log {compoundOutputFileLuid2} \
-t true \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/novaseq/novaseq-
extensions.jar -u {username} -p {password} -e {compoundOutputFileLuid2} \
script:generate_novaseq_sample_sheet \
-i {processURI:v2} \
-c {compoundOutputFileLuid1} \
-r {udf:Reverse Complement Workflow} \
script:generate_novaseq_run_recipe \
-i {stepURI:v2} \
-c {compoundOutputFileLuid0}"
```

You can also update the existing automation by deleting the following code snippet from the first -exp command line:

```
if (step.::Reverse Complement Workflow::.toBoolean()) { fail(::Reverse
Complement Workflow can only be enabled in a Paired End run.::) };
```

4. Select Save.

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NovaSeq 6000 Integration v2.4 Release Notes

These Release Notes describe the key changes to software components for the Clarity LIMS NovaSeq 6000 Integration Package since version 2.3.

This is an optional software update for customers interested in the features available with this release.

If upgrading from a version prior to version 2.3, review the release notes for v2.3 for a list of features and bug fixes introduced in that version.

Compatibility

This version of the integration package is compatible with the following software:

- Clarity LIMS v5.1 and later
- NGS Extensions Package v5.21 and later
- NovaSeq Control Software (NVCS) v1.8.0
- Illumina bcl2fastq v2.20

New Features

Features provided with this release of the integration package are as follows:

- Support for Clarity LIMS v6.0. Clarity LIMS v4.x and v5.0 are not supported.
- Tech refresh to upgrade third party libraries.
- Fixes to address the following Apache log4j vulnerabilities:
  - CVE-2021-44228
  - CVE-2021-45046
  - CVE-2021-45105
  - CVE-2021-44832
- Security bug fixes.

Defects Fixed

- Fixed to allow Reverse Complement Workflow in all cases.
- Fixed the Oracle 4000 max character limit issue. Moved long automation script into a custom field.
- Fixed orientation of Index2 value in generated sample sheet.
- Fixed result discrepancy issue when Calculate Volume automation is run more than once.
- Fixed Calculate Volume automation result file to show only one decimal place.

Known Issues

Blocker alert: When an artifact is removed and queued to another workflow, the "Queued" workflow-stage of the artifact is missing on the API level. Consequently, the recipe request of a NovaSeq run fails due to the missing "Queued" stage.

This issue only affects Clarity LIMS v5.2.1. The issue will be fixed in the upcoming Clarity LIMS v5.2.2 release.

NovaSeq 6000 Integration v2.3 Configuration and User Interaction

The Illumina NovaSeq 6000 Integration Package v2.3 supports the integration of Clarity LIMS to Illumina NovaSeq 6000 instruments.

This user and configuration guide describes the integration and includes information about the preconfigured protocols, steps, automations, installed components, configuration requirements, rules, and constraints.

For instructions on validating and troubleshooting the Illumina NovaSeq 6000 Integration, see the NovaSeq Integration v2.3 Validation & Troubleshooting Guide.

The configuration provided in this integration has been established to support NovaSeq 6000 lab processes. Any configuration changes to protocols or workflows—including renaming protocols, steps, and fields—could break the process.

The integration includes the following functionality:

- Preconfigured NovaSeq 6000 v2.1 workflow that maps to lab protocols and instrument runs.
- Preconfigured NovaSeq Standard (NovaSeq 6000 v2.1) protocol that supports the loading of pooled libraries into a library tube.
- Preconfigured NovaSeq Xp (NovaSeq 6000 v2.1) protocol that supports individual lane loading on the NovaSeq.
- Automated generation of a sample sheet for use with bcl2fastq2 v2.20 analysis software.
- Automatic generation of run recipe file (JSON format) that is automatically uploaded to the sequencing instrument and used to set up and initiate the run.
- Automated tracking of the NovaSeq sequencing run and parsing of run statistics (per run per lane) into Clarity LIMS, including:
  - Progress and metrics of sequencing run
  - Per instrument sequencing runs (tracked as part of the Run ID field value)
  - Sequencing run parameters
  - Real-Time Analysis v3 (RTA3) run directory location and other run-specific information

- Preconfigured NovaSeq Validation Library Prep (NovaSeq 6000 v2.1) workflow used for validation purposes only. The workflow contains a single-step protocol that models the library prep required to produce normalized libraries that are ready for the NovaSeq 6000 v2.1 workflow. For details, see the NovaSeq 6000 Integration v2.3 Validation and Troubleshooting Guide.
- Preconfigured NovaSeq 6000 v2.1 supports NVCS v1.7 with the following new functions:
  - Unique Molecular Identifier (UMI) settings
  - Reverse complement workflow and reverse complement sample sheet generation
  - Supports new NovaSeq reagent kit v1.5
- Preconfigured NovaSeq 6000 v2.1 adds support for NovaSeq SP flow cell.

Information on these features is provided in the following sections.

Prerequisites and Assumptions

It is assumed that samples enter the NovaSeq 6000 v2.1 workflow as normalized libraries. That is, before they are assigned to the workflow the following actions occur:

- Samples have been accessioned into Clarity LIMS.
- Samples have been run through QC and library prep.
- Samples have been normalized, and the value is captured in a field called Normalized Molarity (nM).

For details on sample accessioning, see the Clarity LIMS documentation.

Samples can be assigned to the NovaSeq 6000 v2.1 workflow automatically (using a routing script) or manually from the Projects and Samples dashboard. See *Assigning Samples to Workflows*.

Workflows, Protocols, and Steps

The Illumina NovaSeq 6000 Integration Package v2.3 includes two workflows:

- NovaSeq 6000 v2.1
- NovaSeq Validation Library Prep (NovaSeq 6000 v2.1)

Table 1 lists and describes the protocols and steps included in these workflows.

Table 279 Table 1: Illumina NovaSeq 6000 Integration Package v2.3 Protocols and Steps

Protocol Name	Protocol Purpose	Steps Included in Protocol
NovaSeq Validation Library Prep (NovaSeq 6000 v2.1) workflow		

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Protocol Name	Protocol Purpose	Steps Included in Protocol
NovaSeq Validation Library Prep (NovaSeq 6000 v2.1)	Included for validation purposes only, this protocol models the library prep steps required to advance samples to the Run Format (NovaSeq 6000 v2.1) protocol. The protocol contains a single step: NovaSeq Validation Library Prep (NovaSeq 6000 v2.1). At the end of this step, a routing script sends the samples to the first step of the NovaSeq 6000 v2.1 workflow, which is Define Run Format (NovaSeq 6000 v2.1).	NovaSeq Validation Library Prep (NovaSeq 6000 v2.1)
	NovaSeq 6000 v2.1 workflow	
Run Format (NovaSeq 6000 v2.1)	Allows for the assignment of per sample values for Loading Workflow Type, Normalized Molarity, Flowcell Type, and Final Loading Concentration (pM). • Loading Workflow Type: Select from NovaSeq Standard or NovaSeq Xp. • Normalized Molarity: Enter a value for each sample. • Flowcell Type: Select from options S1, S2, S4, and SP. • Final Loading Concentration (pM): Select from the options 225 (PCR-free workflows) or 400 (Nano workflows). Alternatively, enter a different value. Compares the Normalized Molarity value of each sample with the Minimum Molarity value. Routing script sends samples to the NovaSeq Standard or NovaSeq Xp protocol, according to the selected Loading Workflow Type. Samples with Normalized Molarity less than Minimum Molarity are removed from the workflow.	Define Run Format (NovaSeq 6000 v2.1
NovaSeq Standard (NovaSeq 6000 v2.1	Samples are pooled and added to the library tube in preparation for the NovaSeq run. Sample sheet and run recipe files are generated. Routing script sends library tube to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) protocol.	Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.1) Dilute and Denature (NovaSeq 6000 v2.1)

Protocol Name	Protocol Purpose	Steps Included in Protocol
NovaSeq Xp (NovaSeq 6000 v2.1)	Samples are pooled and added to lanes on the NovaSeq flow cell. The flow cell type is determined by the option selected in the Define Run Format (NovaSeq 6000 v2.1) step. Sample sheet and run recipe files are generated. Samples are queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.1) protocol.	Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.1) Dilute, Denature, and ExAmp (NovaSeq 6000 v2.1) Load to Flowcell (NovaSeq 6000 v2.1)
AUTOMATED - NovaSeq Run (NovaSeq6000 v2.1)	All samples complete the workflow by going through this protocol. This protocol contains one fully automated step. Do not add samples to the Ice Bucket or start the step. The integration does this action automatically.	AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.1)

Validation Workflow

The NovaSeq Validation Library Prep (NovaSeq 6000 v2.1) workflow allows for validation of the system after installation is complete. For details, see the *NovaSeq 6000 Integration v2.3 - Validation and Troubleshooting Guide*.

Step Automations

Table 2 lists the step automations included in the NovaSeq 6000 v2.1 workflow. Automation details are provided in the sections following the table.

For details on the validation workflow, see the *NovaSeq 6000 Integration v2.3 - Validation and Troubleshooting Guide*.

Table 280 Table 2: NovaSeq 6000 v2.1 Workflow Step Automations

Protocol	Step	Automation
1. Run Format (NovaSeq 6000 v2.1)	1. Define Run Format (NovaSeq 6000 v2.1)	• Routing Script • Set Next Step

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Protocol	Step	Automation
2. NovaSeq Standard (NovaSeq 6000 v2.1)	1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.1)	• Calculate Volumes • Set Next Step • Validate Inputs Flowcell Type and Single Pool • Validate Unique Indexes Not used. This functionality is handled by the core Clarity LIMS configuration for pooling.
	2. Dilute and Denature (NovaSeq 6000 v2.1)	• Generate Sample Sheet and Run Recipe • Prepare Files for NovaSeq • Routing Script • Validate Library Tube Barcode • Validate Single Input
3. NovaSeq Xp (NovaSeq 6000 v2.1)	1. Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.1)	• Calculate Volumes • Set Next Step • Validate Inputs Flowcell Type and Single Pool • Validate Unique Indexes Not used. This functionality is handled by the core Clarity LIMS configuration for pooling.
	2. Dilute, Denature, and ExAmp (NovaSeq 6000 v2.1)	• Calculate Volumes • Set Next Step • Validate Inputs Flowcell Type
	3. Load to Flowcell (NovaSeq 6000 v2.1)	• Generate Sample Sheet and Run Recipe • Prepare Files for NovaSeq • Validate Flowcell Barcode • Validate Inputs and Selected Container
4. AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.1)	1. AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.1)	• Parse Run Parameters and Generate Link to Run Folder • Read InterOp Metrics • Set Next Steps • Update Lane number

Configuration Details

The following sections describe the configuration installed with the Illumina NovaSeq 6000 Integration.

The Illumina NovaSeq 6000 Integration Package v2.3 supports Clarity LIMS v4.2 and later.

As of Clarity LIMS v5, the term user-defined field (UDF) has been replaced with custom field in the user interface (the API resource is still called UDF). There are two types of custom fields:

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- Master step fields—Configured on master steps. These fields only apply to the master step on which they are configured, and the steps derived from those master steps.
- Global fields—Configured on entities such as submitted sample, derived sample, measurement, etc. These fields apply to the whole Clarity LIMS system.

Protocol 1: Run Format (NovaSeq 6000 v2.1)

This protocol sets the Loading Workflow Type and allows you to choose the appropriate Flowcell Type and Final Loading Concentration(pM). At the end of the protocol, a routing script sends the normalized libraries to the NovaSeq Xp (NovaSeq 6000 v2.1) protocol.

This protocol contains one step: Define Run Format (NovaSeq 6000 v2.1).

Step 1: Define Run Format (NovaSeq 6000 v2.1)

Step input: NTP (normalized libraries)

Step output: None

The following automations are configured on this step:

- Routing Script
- Set Next Steps

These automations are described in the order in which they are triggered at run time.

Set Next Steps

Automatically triggered on exit of the Record Details screen, this automation does the following actions:

- Sets the next step for samples to REMOVE:

```
nextStep = ::REMOVE::
```

- Calculates the Minimum Molarity using the following formula:

```
input.::Minimum Molarity (nM):: = (5 * input.::Final Loading Concentration
(pM)::)/1000
```

- Checks Normalized Molarity value. For samples with no Normalized Molarity value (ie, an empty value, not including 0), the automation generates an error message stating that the field cannot be empty:

```
if (!input.hasValue(::Normalized Molarity (nM)::)) { fail(::The Normalized
Molarity cannot be empty.::) ; }
```

- Compares the Normalized Molarity value of each sample with the Minimum Molarity value.
- If Normalized Molarity value is lower than the Minimum Molarity value, sets the Loading Workflow Type of the sample to [Remove from workflow] and records a message in the Warning field:

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```
else if (input.::Normalized Molarity (nM):: < input.::Minimum Molarity (nM)::) {
input.::Warning:: = ::The Normalized Molarity is too low.:: ; input.::Loading
Workflow Type:: = ::[Remove from workflow]:: ; } else { input.::Warning:: =
::n/a:: }
```

At this point there are two options:

- Correct the Normalized Molarity value on the Record Details screen. Also, edit the Loading Workflow Type field and set it to NovaSeq Standard or NovaSeq Xp, as applicable.
- Complete the protocol without correcting the Normalized Molarity value. In this case, those samples are removed from the workflow.

Routing Script

Automatically triggered on exit of the step, this automation invokes the changeWorkflow script, which routes step inputs appropriately.

- Samples with Loading Workflow Type field value of NovaSeq Standard are routed to the NovaSeq 6000 v2.1 workflow and queued for the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.1) step.
- Samples with Loading Workflow Type field value of NovaSeq Xp are routed to the NovaSeq 6000 v2.1 workflow and queued for the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.1) step.

The default automation command line is as follows.

```
bash -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -u {username} -p {password} -i {stepURI:v2} -l
{compoundOutputFileLuid0} script:changeWorkflow \\--FIELD_NAME 'Loading Workflow
Type' \--FIELD_VALUE 'NovaSeq Standard' \--WORKFLOW 'NovaSeq 6000 v2.1' \--STEP
'Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.1)' \--INPUTS_OR_OUTPUTS
'INPUTS' \\--FIELD_NAME 'Loading Workflow Type' \--FIELD_VALUE 'NovaSeq Xp' \--
WORKFLOW 'NovaSeq 6000 v2.1' \--STEP 'Make Bulk Pool for NovaSeq Xp (NovaSeq
6000 v2.1)' \--INPUTS_OR_OUTPUTS 'INPUT
```

Step UDFs/Custom Fields

There are two fields defined on the Define Run Format (NovaSeq 6000 v2.1) step.

These fields are shown in the Clarity LIMS v5 Configuration area (Custom Fields > Master Step Fields tab). See Table 3 for Clarity LIMS v4.2 and Clarity LIMS v5 field configuration details.

Table 281 Table 3: Define Run Format (NovaSeq 6000 v2.1) Step UDF/Custom Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Comment	Multiline Text	• Required: No • Read Only: No	None
Instruction	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	• Default: Add Flowcell Type and Loading Workflow Type below
Flowcell Type	Single-line Text (v4) Text (v5)	• Required: Yes • Read Only: No • Custom Entries: No	Hidden, Not in use
Loading Workflow Type	Single-line Text (v4) Text (v5)	• Required: Yes • Read Only: No • Custom Entries: No	Hidden, Not in use

Global UDFs/Custom Fields

Table 4 lists the global UDFs/custom fields that are configured to display on the Define Run Format (NovaSeq 6000 v2.1) step.

Table 282 Table 4: Global UDF/Custom Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Configured on Analyte in Clarity LIMS v4 and Derived Sample in Clarity LIMS v5:			
Adjusted Per Sample Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Final Loading Concentration (pM)	Numeric (v4) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 225 • 400 • Decimal Places Displayed: 0
Flowcell Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• S1 • S2 • S4 • SP

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Loading Workflow Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
Minimum Molarity (nM)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2
Normalized Molarity (nM)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2
Per Sample Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Warning	Single-line Text (v4) Text Dropdown (v5)	• Required: No • Read Only: Yes • Custom Entries: Yes	• The Normalized Molarity (nM) is too low. • Not applicable

User Interaction

1. On the Record Details screen in the Sample Details table, complete the following fields (values can vary across samples):
   - Loading Workflow Type: Select NovaSeq Standard or NovaSeq Xp from the drop-down list.
   - Flowcell Type: Select S1, S2, S4, or SP from the drop-down list.
   - Final Loading Concentration (pM): Select from the two preset options—225 (for PCR-free workflows) or 400 (for Nano workflows)—or enter a different value.
   - Normalized Molarity: Enter a value for each sample.
2. Select Next Steps. This selection triggers the Next Steps automation, which does the following actions:
   - Sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step successfully.
   - Calculates the Minimum Molarity.
   - Checks Normalized Molarity value. For samples with no Normalized Molarity value (ie, an empty value, not including 0), the automation generates an error message stating that the field cannot be empty.
   - Compares the Normalized Molarity value of each sample with the Minimum Molarity value.

3. On the Assign Next Steps screen:
   - In the Sample Details table, the Next Step for all samples is prepopulated with Remove from workflow—regardless of the Loading Workflow Type.

     > Do not change this value! If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.

   - For samples whose Normalized Molarity value is lower than the Minimum Molarity value, the Loading Workflow Type is set to Remove from workflow and a message is recorded in the Warning field for the sample.

   At this point there are two options:
   - Return to the Record Details screen and adjust the Normalized Molarity value so that it equals or exceeds the Minimum Molarity value. Also, set the Loading Workflow Type to NovaSeq Standard.
   - Complete the protocol without correcting the Normalized Molarity value. In this case, the samples in question are removed from the Clarity LIMS workflow.
4. Select Finish Step. The Routing Script automation is triggered:
   - Samples whose Loading Workflow Type is set to Remove from workflow (ie, where Normalized Molarity value is lower than the Minimum Molarity) are removed from the Clarity LIMS workflow.
   - Samples whose Loading Workflow Type is set to NovaSeq Standard are routed to the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.1) step. This step is the first of two steps in the NovaSeq Standard (NovaSeq 6000 v2.1) protocol.
   - Samples whose Loading Workflow Type is set to NovaSeq Xp are routed to the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.1) step. This step is the first of three steps in the NovaSeq Xp (NovaSeq 6000 v2.1) protocol.

Protocol 2: NovaSeq Standard (NovaSeq 6000 v2.1)

Samples are routed to this protocol if the Loading Workflow Type value is set to NovaSeq Standard. Samples are pooled and added to a library tube in preparation for the NovaSeq run.

At the end of this protocol, a routing script sends the library tube to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.1) protocol.

This protocol contains two steps:

1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.1)
2. Dilute and Denature (NovaSeq 6000 v2.1)

Step 1: Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.1)

In this step, manually place libraries into a single pool. Resuspension buffer and reagents are added.

> Only create one pool per step.

Step input: NTP (normalized libraries)

Step output: Bulk pool

The following automations are configured on the step:

- Calculate Volumes
- Set Next Step
- Validate Inputs Flowcell Type and Single Pool
- Validate Unique Indexes (Not used. This functionality is handled by the core Clarity LIMS configuration for pooling.)
- Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.1) automation configuration—Clarity LIMS v4
- Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.1) automation configuration—Clarity LIMS v5

These automations are described in the order in which they are triggered at run time.

Validate Inputs Flowcell Type and Single Pool

Automatically triggered on exit of the Pooling screen, this automation checks the following information:

- All samples in the pool have the same Flowcell Type assigned to them.
- Only one pool has been created.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/novaseq/novaseq-extensions.jar script:validate_flowcell_for_input_pools -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -validateSingleOutput true -poolType bulk
```

Calculate Volumes

Automatically triggered when the Calculate Volumes button on the Record Details screen is selected, this automation does the following actions:

- Calculates the number of samples in the pool:

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: + 1
```

- Sets the value of the Bulk Pool Volume (µl) field, based on the selected Flowcell Type:

```
if (input.::Flowcell Type:: == ::S1::) { step.::Bulk Pool Volume (µl):: = step.::Number of Flowcells to Sequence:: * 100 + 30 }; if (input.::Flowcell Type:: == ::S2::) { step.::Bulk Pool Volume (µl):: = step.::Number of Flowcells to Sequence:: * 150 + 30 }; if (input.::Flowcell Type:: == ::S4::) { step.::Bulk Pool Volume (µl):: = step.::Number of Flowcells to Sequence:: * 310 + 30 };
```

- Calculates the Per Sample Volume (µl) to be added to the pool:

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```
input.::Per Sample Volume (µl):: = ( ( (input.::Final Loading Concentration
(pM):: * 5 / 1000) / input.::Normalized Molarity (nM):: ) * step.::Bulk Pool
Volume (µl):: ) / step.::Number of Samples in Pool::
```

> To make sure accurate pipetting of each sample in a pool for sequencing, the Per Sample Volume (µl) value must be equal to or higher than the Minimum Per Sample Volume (µl). The default value is set at 5, which can be edited.
>
> Assuming the default Minimum Per Sample Volume (µl) value of 5, for a given batch:
>
> 1. If the smallest Per Sample Volume (µl) value is less than 5, Clarity LIMS automatically assigns a value of 5 to the Adjusted Per Sample Volume (µl) field.
> 2. The Clarity LIMS then adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

- Calculates the Total Sample Volume (µl) field value:

```
step.::Total Sample Volume (µl):: = step.::Total Sample Volume (µl):: +
input.::Adjusted Per Sample Volume (µl)::
```

- If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) field value:

```
if (step.::Total Sample Volume (µl):: >= step.::Bulk Pool Volume (µl)::)
{output.::RSB Volume (µl):: = 0} else {output.::RSB Volume (µl):: = step.::Bulk
Pool Volume (µl):: - step.::Total Sample Volume (µl)::} ;
```

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs:

```
output.::Flowcell Type::= input.::Flowcell Type::;output.::Loading Workflow
Type::= input.::Loading Workflow Type::;
```

- Sets the Volume of Pool to Denature (µl) value and calculates NaOH Volume (µl) and Tris-HCl Volume (µl) values, based on the Flowcell Type:

```
if ( input.::Flowcell Type:: == ::S1:: ) { output.::Volume of Pool to Denature
(µl):: = 100 ; output.::NaOH Volume (µl):: = 25.00 ; output.::Tris-HCl Volume
(µl):: = 25.00 } ; if ( input.::Flowcell Type:: == ::S2:: ) {output.::Volume of
Pool to Denature (µl):: = 150 ; output.::NaOH Volume (µl):: = 37.50 ;
output.::Tris-HCl Volume (µl):: = 37.50 } ; if ( input.::Flowcell Type:: ==
::S4:: ) { output.::Volume of Pool to Denature (µl):: = 310 ; output.::NaOH
Volume (µl):: = 77.50 ; output.::Tris-HCl Volume (µl):: = 77.50 }
```

- Uses the `NovaSeq_Standard_Bulk_Pool1.csv`, `NovaSeq_Standard_Bulk_Pool2.csv`, and `NovaSeq_Standard_Bulk_Pool3.csv` template files to generate a single CSV file containing information about the pool and the samples it contains. The generated file is available for download on the Step Setup screen of the following step: Dilute and Denature (NovaSeq 6000 v2.1).

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```
script:driver_file_generator \ -t
/opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Standard_Bulk_Pool1.csv \
-o 1.csv \ script:driver_file_generator \ -t
/opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Standard_Bulk_Pool2.csv \
-o 2.csv \ script:driver_file_generator \ -t
/opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Standard_Bulk_Pool3.csv \
-o 3.csv \&& cat 1.csv 2.csv 3.csv > {compoundOutputFileLuid0}.csv
```

Set Next Step

Automatically triggered on exit of the Record Details screen, this automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol—Dilute and Denature (NovaSeq 6000 v2.1):

```
nextStep = ::ADVANCE::
```

Step UDFs/Custom Fields

There are five fields defined on the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.1) step.

These fields are shown in the Clarity LIMS v5 Configuration area (Custom Fields > Master Step Fields tab). See Table 5 for Clarity LIMS v4 and Clarity LIMS v5 field configuration details.

Table 283 Table 5: Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.1) Step UDF/Custom Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Bulk Pool Volume (µl) (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2
Number of Flowcells to Sequence	Numeric	• Required: Yes • Read Only: No	• Range From: 1 to 10 • Decimal Places Displayed: 0
Total Sample Volume (µl) (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Default: 0 • Decimal Places Displayed: 2
Minimum Per Sample Volume (µl)	Numeric	• Required: Yes • Read Only: No	• Default: 5 • Decimal Places Displayed: 2

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Number of Samples in Pool (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Default: 0 • Decimal Places Displayed: 0

Global UDFs/Custom Fields

Table 6 lists the global UDFs/custom fields that are configured to display on the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.1) step.

Table 284 Table 6: Global UDF/Custom Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Configured on Analyte in Clarity LIMS v4 and Derived Sample in Clarity LIMS v5:			
Flowcell Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• S1 • S2 • S4 • SP
Loading Workflow Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
NaOH Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
RSB Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Tris-HCl Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Volume of Pool to Denature (µl) Used in Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.1) step only. Displays on Record Details screen and in the generated CSV file.	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0

User Interaction

1. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucket and select View Ice Bucket.
2. On the Ice Bucket screen, select Begin Work.
3. On the Pooling screen:
   a. Create a pool by dragging samples into the Pool Creator.
   b. Name the pool or accepts the default name (Pool #1).
   c. Select Record Detail.
   On exit from the Pooling screen, the Validate Inputs Flowcell Type and Single Pool is triggered. The automation checks that all samples in the pool have been assigned the same Flowcell Type and only one pool has been created.
4. On the Record Details screen, the Step Details area contains two required fields and one optional field:
   - Number of Flowcells to Sequence—The value entered in this field is used in volume calculations, to make sure that volumes are sufficient for the number of times the pool is sequenced.
   - Minimum Per Sample Volume (µl)—In many applications, and particularly where robotics are used, a minimum sample volume is required. The value in this field is used to calculate how much of each sample is included in the pool. The field is prepopulated with the configured default value (5 µl). It can be edited if necessary.
   - % PhiX (2.5 nM) Spike-In—The value in this field is used to calculate the volume of PhiX v3 control to be included in the pool for the given percentage of spike-in. The field is optional and can be edited if necessary.
5. In the Sample Details table, select the pool icon to view details on the pool composition.
6. Select Calculate Volumes. This selection triggers the Calculate Volumes automation, which performs the following volume calculations based on the selected Flowcell Type:

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- Calculates Bulk Pool Volume (µl) value.
- Calculates PhiX Volume (µl) value if % PhiX (2.5 nM) Spike-In has value.
- Calculates the Per Sample Volume (µl) value to be added to the pool.
- Calculates the Total Sample Volume (µl) value.
- If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) value.
- Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
- Populates the Volume of Pool to Denature (µl), NaOH Volume (µl), and Tris-HCl Volume (µl) columns of the Sample Details table. (Values are set by a script and are not editable.)
- Generates the Calculation File (CSV) and attaches it to the step. This file contains volume information about the pool and the individual samples it contains. Select the file to download it, then open it in Excel.

7. Select Next Steps. This selection triggers the Set Next Step automation. This automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol: Dilute and Denature (NovaSeq 6000 v2.1).
   On the Assign Next Steps screen, the next step is already set to Dilute and Denature (NovaSeq 6000 v2.1).
8. Select Finish Step. At the end of this step, the pool of samples automatically advances to the Dilute and Denature (NovaSeq 6000 v2.1) step.

Step 2: Dilute and Denature (NovaSeq 6000 v2.1)

In this step, pooled samples are denatured and diluted by the addition of NaOH, Tris-HCl, and Resuspension Buffer (RSB). Samples are manually placed into the library tube that is used in the NovaSeq run. In addition, this step generates the sample sheet file and the run recipe (*json file) needed to start the NovaSeq run.

Step input: Bulk pool

Step output: Library tube

The following automations are configured on the step:

- Generate Sample Sheet and Run Recipe
- Prepare Files for NovaSeq
- Routing Script
- Validate Library Tube Barcode
- Validate Single Input
- Dilute and Denature (NovaSeq 6000 v2.1) automation configuration—Clarity LIMS v4
- Dilute and Denature (NovaSeq 6000 v2.1) automation configuration—Clarity LIMS v5

These automations are described in the order in which they are triggered at run time.

Validate Single Input

Automatically triggered at the beginning of the step, this automation checks that there is only one container input to the step.

```
script:validateSampleCount -min 1 -max 1
```

Validate Library Tube Barcode

Automatically triggered on exit of the Placement screen, this automation validates the library tube barcode to make sure it conforms to the barcode mask [A-Za-z]{2}[0-9]{7}-[A-Za-z]{3}:

```
if (!output.container.name.matches(::[A-Za-z]{2}[0-9]{7}-[A-Za-z]{3}::)) { fail
(::Invalid Library Tube Barcode. Please verify and try again.::) }
```

Generate Sample Sheet and Run Recipe

Automatically triggered when a button on the Record Details screen is selected, this automation does the following actions:

- Copies the Flowcell Type from the step input to the step output:

```
output.::Flowcell Type:: = input.::Flowcell Type::
```

- Copies the Flowcell Type from the step input to the Run Mode field (hidden):

```
step.::Run Mode:: = input.::Flowcell Type::
```

- Copies the Loading Workflow Type values from the step inputs to the step outputs:

```
output.::Loading Workflow Type:: = input.::Loading Workflow Type::;
```

- Validates the parameters entered on the Record Details screen:
  - Experiment Name can only contain alphanumeric, dash, or underscore characters. Spaces are not permitted.
  - When Workflow Type is No Index, Index Read 1 must be zero. For any other Workflow Type, Index Read 1 must be greater than zero.

```
if(!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) { fail(::Experiment
Name contains prohibited characters. Allowed characters are: a-z, A-Z, 0-9, -,
and _::)};if(step.::Workflow Type::== ::No Index::) { if(step.::Index Read 1::!=
0) { fail(::Index Read 1 must be 0 if the Workflow Type is No Index.::) }} else
{ if(step.::Index Read 1::== 0) { fail(::Index Read 1 must be greater than 0 if
the Workflow Type is ::+ step.::Workflow Type::+ ::.::) } }
```

- When Paired End is True, Index Read 1 and Index Read 2 must be greater than zero.
- When Paired End is False, Index Read 2 must be zero, Reverse Complement Workflow must be false, UMI - Read 2 Length must not have a value, and UMI - Read 2 Start from Cycle must not have a value.

```
if (step.::Paired End::.toBoolean()) { if (step.::Read 1 Cycles:: == 0 ||
step.::Read 2 Cycles:: == 0) { fail(::Read 1 Cycles and Read 2 Cycles must be
greater than 0 if Paired End is True.::); } } else { if (step.::Read 2 Cycles::
!= 0) { fail(::Read 2 Cycles must be 0 if Paired End is False.::); } if
(step.'Reverse Complement Workflow'.toBoolean()) { fail(::Reverse Complement
Workflow can only be enabled in a Paired End run.::); } if (step.hasValue(::UMI
- Read 2 Length::) || step.hasValue(::UMI - Read 2 Start From Cycle::)) { fail
(::UMI - Read 2 Length and UMI - Read 2 Start From Cycle cannot be defined if
Paired End is False.::); } }
```

- Validates allowed read cycles to be not greater than 151 if the Flowcell Type is not SP.
  - When Workflow Type is Dual Index or Custom, Index Read 2 is set to the same as Index Read 1.

```
if (step.::Workflow Type:: == ::No Index:: || step.::Workflow Type:: == ::Single
Index::) { step.::Index Read 2:: = 0 } else { step.::Index Read 2:: =
step.::Index Read 1:: }
```

  - When Paired End is true, Index Read 1 and Index Read 2 must be greater than zero.
  - When Paired End is false, Index Read 2 must be zero, Reverse Complement Workflow must be false, UMI - Read 2 Length must not have a value, and UMI - Read 2 Start from Cycle must not have a value.

```
if (step.'Paired End'.toBoolean()) {if (step.'Read 1 Cycles' == 0 || step.'Read
2 Cycles' == 0) {fail('Read 1 Cycles and Read 2 Cycles must be greater than 0 if
Paired End is True.');}
```

```
} else {if (step.'Read 2 Cycles' != 0) {fail('Read 2 Cycles must be 0 if Paired
End is False.');}if (step.'Reverse Complement Workflow'.toBoolean()) {fail
('Reverse Complement Workflow can only be enabled in a Paired End run.');}if
(step.hasValue('UMI - Read 2 Length') || step.hasValue('UMI - Read 2 Start From
Cycle')) {fail('UMI - Read 2 Length and UMI - Read 2 Start From Cycle cannot be
defined if Paired End is False.');}}
```

- Validates allowed read cycles to be not greater than 151 if the Flowcell Type is not SP.

```
if (step.hasValue(::UMI - Read 1 Length::) && !step.hasValue(::UMI - Read 1
Start From Cycle::)) { fail(::UMI - Read 1 Start From Cycle must be greater than
0 if UMI - Read 1 Length is greater than 0.::); } if (!step.hasValue(::UMI -
Read 1 Length::) && step.hasValue(::UMI - Read 1 Start From Cycle::)) { fail
(::UMI - Read 1 Length must be greater than 0 if UMI - Read 1 Start From Cycle
is greater than 0.::); } if (step.hasValue(::UMI - Read 2 Length::) &&
!step.hasValue(::UMI - Read 2 Start From Cycle::)) { fail(::UMI - Read 2 Start
From Cycle must be greater than 0 if UMI - Read 2 Length is greater than 0.::);
} if (!step.hasValue(::UMI - Read 2 Length::) && step.hasValue(::UMI - Read 2
Start From Cycle::)) { fail(::UMI - Read 2 Length must be greater than 0 if UMI
- Read 2 Start From Cycle is greater than 0.::); } if (!step.hasValue(::UMI -
Read 1 Length::) && step.hasValue(::UMI - Read 2 Length::)) { fail(::UMI - Read
```


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```
1 Length must be greater than 0 if UMI - Read 2 Length is greater than 0.::); }
if (!step.hasValue(::UMI - Read 1 Start From Cycle::) && step.hasValue(::UMI -
Read 2 Start From Cycle::)) { fail(::UMI - Read 1 Start From Cycle must be
greater than 0 if UMI - Read 2 Start From Cycle is greater than 0.::); }
```

- Sets the next step for samples to REMOVE:

```
nextStep = ::REMOVE::
```

- Generates the sample sheet and the run recipe file and attaches them to the step. For details on how the files are generated and their contents, see the *Sample Sheet and Run Recipe File Generation* section.

Prepare Files for NovaSeq

Automatically triggered on exit of the Record Details screen, this automation invokes the `copy_attachments_to_network_folder` script, which is included in the `novaseq-remote-extensions.jar` file. This script places the generated sample sheet and run recipe (*json file) into the appropriate folder on the NAS, where the instrument uses them to start the run.

For more information, see the *Sample Sheet and Run Recipe File Generation* section.

Routing Script

Automatically triggered on exit of the step, this automation invokes the changeWorkflow script, which routes step outputs to the NovaSeq 6000 v2.1 workflow, and queues them for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.1) step.

The default automation command line is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -u {username} -p {password} -i {stepURI:v2} -l
{compoundOutputFileLuid2} script:changeWorkflow \
\
--FIELD_NAME 'N/A' \
--FIELD_VALUE 'N/A' \
--WORKFLOW 'NovaSeq 6000 v2.0' \
--STEP 'AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0)' \
--INPUTS_OR_OUTPUTS 'OUTPUTS'"
```

Step UDFs/Custom Fields

There are 14 fields defined on the Dilute and Denature (NovaSeq 6000 v2.1) step. These fields are required for sample sheet and JSON file generation.

These fields are shown in the Clarity LIMS v5 Configuration area (Custom Fields > Master Step Fields tab). See Table 7 for Clarity LIMS v4 and Clarity LIMS v5 field configuration details.

Table 285 Table 7: Dilute and Denature (NovaSeq 6000 v2.1) Step UDF/Custom Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
BaseSpace Sequence Hub Configuration	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• Not Used • Run Monitoring Only • Run Monitoring and Storage
Experiment Name	Single-line Text (v4) Text (v5)	• Required: Yes • Read Only: No	Not applicable
Index Read 1	Numeric (v4) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 0 • 6 • 8 • Range From: 0 to 20 • Decimal Places Displayed: 0
Index Read 2	Numeric (v4) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 0 • 6 • 8 • Range From: 0 to 20 • Decimal Places Displayed: 0
Paired End	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• True • False

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Read 1 Cycles	Numeric (v4) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 251 • 151 • 101 • 51 • Range From: 1 to 251 • Decimal Places Displayed: 0
Read 2 Cycles	Numeric (v4) Numeric (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 251 • 151 • 101 • 51 • Range From: 1 to 251 • Decimal Places Displayed: 0
Reverse Complement Workflow	Checkbox (v4) Toggle Switch (v5)	• Required: Yes • Read Only: No	• Default: Yes
Run Mode (not displayed in user interface)	Single-line Text (v4) Text Dropdown (v5)	• Required: No • Read Only: Yes • Custom Entries: No	• S1 • S2 • S4
Sample Sheet Path	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable
UMI — Read 1 Length	Numeric (v4) Numeric (v5)	• Required: No • Read Only: No	• Range From: 1 • Decimal Places Displayed: 0

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
UMI — Read 1 Start From Cycle	Numeric (v4) Numeric (v5)	• Required: No • Read Only: No	• Range From: 1 • Decimal Places Displayed: 0
UMI — Read 2 Length	Numeric (v4) Numeric (v5)	• Required: No • Read Only: No	• Range From: 1 • Decimal Places Displayed: 0
UMI — Read 2 Start From Cycle	Numeric (v4) Numeric (v5)	• Required: No • Read Only: No	• Range From: 1 • Decimal Places Displayed: 0
Use Custom Index Read 1 Primer (For Clarity LIMS v5.0.5 and later, see Configuration Update documentation.)	Check Box (v4.2) Toggle Switch (v5)	• Required: No • Read Only: No	• None Set
Use Custom Read 1 Primer (For Clarity LIMS v5.0.5 and later, see Configuration Update documentation.)	Check Box (v4.2) Toggle Switch (v5)	• Required: No • Read Only: No	• None Set
Use Custom Read 2 Primer (For Clarity LIMS v5.0.5 and later, see Configuration Update documentation.)	Check Box (v4.2) Toggle Switch (v5)	• Required: No • Read Only: No	• None Set
Workflow	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: Yes	• GenerateFASTQ
Workflow Type	Single-line Text (v4.2) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• No Index • Single Index • Dual Index • Custom

Global UDFs/Custom Fields

Table 8 lists the global UDFs/custom fields that are configured to display on the Dilute and Denature (NovaSeq 6000 v2.1) step.

Table 286 Table 8: Global UDF/Custom Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Configured on Analyte in Clarity LIMS v4 and Derived Sample in Clarity LIMS v5:			
Flowcell Type	• Single-line Text (v4) • Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• S1 • S2 • S4
Loading Workflow Type	• Single-line Text (v4) • Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User interaction

1. On the Placement screen:
   a. Drag the pool into the library tube in the Placed Samples area.
   b. Scan or types the barcode of the library tube into the Library Tube field.
   c. Select Record Details.

   On exit of the Placement screen, the Validate Library Tube Barcode automation checks that the library tube barcode conforms to the barcode mask [A-Za-z]{2}[0-9]{7}-[A-Za-z]{3}. If not, an error message displays.
2. On the Record Details screen:
   - The Reagent Lot Tracking section tracks the NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. Select from the active lots displayed in each drop-down list.

     To make reagent lots available for selection in the Reagent Lot Tracking section, configure and activate them. Do this step on the Reagents and Controls screen (Clarity LIMS v4) or the Configuration > Consumables screen (Clarity LIMS v5).

   - The fields displayed in the Step Details section are used to generate the sample sheet and run recipe files. Some of these fields are autopopulated and some must be completed manually (see Table 9 for details).

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Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.
BaseSpace Sequence Hub Configuration	Select from preset options: Not Used, Run Monitoring Only, or Run Monitoring and Storage.
Workflow	Automatically populated with the preset GenerateFASTQ.
Workflow Type	Select from preset options: No Index, Single Index, and Dual Index.
Index Read 1	Select from preset option: 0, 6, or 8—or types a value between 0 and 20.
Index Read 2	Select from preset option: 0, 6, or 8—or types a value between 0 and 20.
Paired End	Select from preset options: True or False.
Read 1 Cycles	Select from preset options: 251, 151, 101, or 51—or types a value between 1 and 151.
Read 2 Cycles	Select from preset options: 251, 151, 101, or 51—or types a value between 1 and 151.
Use Custom Read 1 Primer	Select if applicable.
Use Custom Read 2 Primer	Select if applicable.
Use Custom Index Read 1 Primer	Select if applicable.
Reverse Complement Workflow	Select Yes (by default) for v1.5 reagents in NovaSeq Control Software v1.7 and onwards. Select No for v1 reagents.
UMI — Read 1 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 1. Leave blank if not applicable.
UMI — Read 2 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 2. Leave blank if not applicable.
UMI — Read 1 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.
UMI — Read 2 Start From Cycle	Enter the cycle number that Read 2 starts from. Leave blank if not applicable.
Sample Sheet Path	Automatically populated after the sample sheet has been generated.

3. Select Generate Sample Sheet and Run Recipe. This selection triggers the automation script, which does the following actions:
   - Generates the sample sheet and JSON files and attaches them to the placeholders in the Files area of the Record Details screen.
   - Populates the Sample Sheet Path field with the path to the sample sheet file.
   - If applicable, populates the Index Read 2 and Read 2 Cycles fields.
   - Sets the value of the next step to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step successfully.
4. Select Next Steps. This selection triggers the Prepare Files for NovaSeq automation, which copies the sample sheet and run recipe files to the location specified during installation.
5. On the Assign Next Steps screen, the Next Step for all samples is prepopulated with Remove from workflow.

   Do not change this value! If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.

6. Select Finish Step to trigger the Routing Script automation and route samples to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.1) protocol.
   In Lab View, the pool of samples is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.1) step.

At this point in the workflow, the user interaction ends. The integration automatically starts the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.1) step and data from the run is parsed back into Clarity LIMS.

Protocol 3: NovaSeq Xp (NovaSeq 6000 v2.1)

Samples are routed to this protocol if the Loading Workflow Type value is set to NovaSeq Xp.

Samples are pooled and added to lanes on the NovaSeq flow cell type selected in the Define Run Format (NovaSeq 6000 v2.1) step. Resuspension buffer is added.

At the end of the protocol, a routing script sends the flow cell to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.1) protocol.

This protocol contains three steps:

1. Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.1)
2. Dilute, Denature, and ExAmp (NovaSeq 6000 v2.1)
3. Load to Flowcell (NovaSeq 6000 v2.1)

Step 1: Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.1)

In this step, manually place libraries into a pool.

Only create one pool per step.

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Step input: NTP (normalized libraries)

Step output: Bulk pool

The following automation is configured on the step:

- Calculate Volumes
- Set Next Step
- Validate Inputs Flowcell Type and Single Pool
- Validate Unique Indexes (Not used. This functionality is handled by the core Clarity LIMS configuration for pooling.)
- Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.1) automation configuration—Clarity LIMS v4
- Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.1) automation configuration—Clarity LIMS v5

These automations are described in the order in which they are triggered at run time.

Validate Inputs Flowcell Type and Single Pool

Automatically triggered on exit of the Pooling screen, this automation checks the following information:

- All samples in the pool have the same Flowcell Type assigned to them.
- Only one pool has been created.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/novaseq/novaseq-extensions.jar script:validate_flowcell_for_input_pools -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -validateSingleOutput true
```

Calculate Volumes

Automatically triggered when the Calculate Volumes button on the Record Details screen is selected, this automation:

- Calculates the number of samples in the pool:

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: + 1
```

- Sets the value of the Bulk Pool Volume (µl) and PhiX Volume (µl) field, based on the selected Flowcell Type:

```
step.::PhiX Volume (µl):: = !step.hasValue(::% PhiX (0.25nM) Spike-In::) ? 0 : step.::% PhiX (0.25nM) Spike-In::;
if (input.::Flowcell Type:: == ::SP::) {
step.::Bulk Pool Volume (µl):: = step.'Number of Lanes to Sequence' * 18 + 30;
step.::PhiX Volume (µl):: *= 1.9;
}
if (input.::Flowcell Type:: == ::S1::) {
step.::Bulk Pool Volume (µl):: = step.'Number of Lanes to Sequence' * 18 + 30;
step.::PhiX Volume (µl):: *= 1.9;
```

```
}
if (input.::Flowcell Type:: == ::S2::) {
step.::Bulk Pool Volume (µl):: = step.'Number of Lanes to Sequence' * 22 + 30;
step.::PhiX Volume (µl):: *= 1.9;
}
if (input.::Flowcell Type:: == ::S4::) {
step.::Bulk Pool Volume (µl):: = step.'Number of Lanes to Sequence' * 30 + 30;
step.::PhiX Volume (µl):: *= 2.2;
}
if (step.::PhiX Volume (µl):: == 0) {
step.::PhiX Volume (µl):: = '';
}
```

- Calculates the Per Sample Volume (µl) to be added to the pool:

```
input.::Per Sample Volume (µl)::= (((input.::Final Loading Concentration (pM)::*
5/ 1000) / input.::Normalized Molarity (nM)::) * step.::Bulk Pool Volume (µl)::)
/ step.::Number of Samples in Pool::
```

> To make sure accurate pipetting of each sample in a pool for sequencing, the Per Sample Volume (µl) value must be equal to or higher than the Minimum Per Sample Volume (µl). The default value set at 5 and can be edited.
>
> Assuming the default Minimum Per Sample Volume (µl) value of 5, for a given batch:
>
> 1. If the smallest Per Sample Volume (µl) value is less than 5, Clarity LIMS automatically assigns a value of 5 to the sample's Adjusted Per Sample Volume (µl) field.
> 2. Clarity LIMS then adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

- Calculates the Total Sample Volume (µl) field value:

```
step.::Total Sample Volume (µl):: = step.::Total Sample Volume (µl):: +
input.::Adjusted Per Sample Volume (µl)::
```

- If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) field value:

```
if (step.::Total Sample Volume (µl):: >= step.::Bulk Pool Volume (µl)::)
{output.::RSB Volume (µl):: = 0} else {output.::RSB Volume (µl):: = step.::Bulk
Pool Volume (µl):: - step.::Total Sample Volume (µl)::} ;
```

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs:

```
output.::Flowcell Type::= input.::Flowcell Type::;output.::Loading Workflow
Type::= input.::Loading Workflow Type::;
```


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- Uses the `NovaSeq_Xp_Bulk_Pool.csv` and `NovaSeq_Xp_Bulk_Pool2.csv` template files to generate a single CSV file containing information about the bulk pool and the samples it contains. The generated file is available for download on the Step Setup screen of the following step—Dilute, Denature, and ExAmp (NovaSeq 6000 v2.0).

Set Next Step

Automatically triggered on exit of the Record Details screen, this automation does the following actions:

- Copies the Flowcell Type values from the step inputs to the step outputs.
- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ; nextStep = ::ADVANCE::
```

Step UDFs/Custom Fields

There are five fields defined on the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.1) step.

These fields are shown in the Clarity LIMS v5 Configuration area (Custom Fields > Master Step Fields tab). See Table 10 for Clarity LIMS v4.2 and Clarity LIMS v5 field configuration details.

Table 287 Table 10: Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.1) Step UDF/Custom Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
% PhiX (0.25nM) Spike-In	Numeric	• Required: No • Read Only: No	• Range: 0 to 100 • Decimal Places Displayed: 0
Bulk Pool Volume (µl) (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2
Minimum Per Sample Volume (µl)	Numeric	• Required: Yes • Read Only: No	• Default: 5 • Decimal Places Displayed: 2
Number of Lanes to Sequence	Numeric	• Required: Yes • Read Only: No	• Decimal Places Displayed: 0
Number of Sample in Pool (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Default: 0 • Decimal Places Displayed: 0

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
PhiX Volume (µl) (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2
Total Sample Volume (µl) (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Default: 0 • Decimal Places Displayed: 0

Global UDFs/Custom Fields

Table 11 lists the global UDFs/custom fields that are configured to display on the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.1) step.

Table 288 Table 11: Global UDF/Custom Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
RSB Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Flowcell Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• S1 • S2 • S4 • SP
Loading Workflow Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User Interaction

1. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucket and select View Ice Bucket.
2. On the Ice Bucket screen, select Begin Work.
3. On the Pooling screen:
   a. Drag samples into the Pool Creator to create a pool.
   b. Name the pool or accepts the default name (Pool #1).

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c. Select Record Details.
On exit from the Pooling screen, the Validate Inputs Flowcell Type and Single Pool is triggered. The automation checks that all samples in the pool have been assigned the same Flowcell Type and only one pool has been created.

4. On the Record Details screen, the Step Details area contains two required fields and one option field:
   - Number of Lanes to Sequence—The value entered in this field is used in volume calculations to make sure that volumes are sufficient for the number of times the pool is sequenced.
   - Minimum Per Sample Volume (µl)—In many applications, and particularly where robotics are used, a minimum sample volume is required. The value in this field is used to calculate how much of each sample is included in the pool. The field is prepopulated with the configured default value (5 µl). It can be edited if necessary.
   - % PhiX (0.25nM) Spike-In—The value in this field is used to calculate the volume of PhiX v3 control to be included in the pool for the given percentage of spike-in. The field is optional and can be edited if needed.
5. In the Sample Details table, select the pool icon to view details on the pool composition.
6. Select Calculate Volumes. This selection triggers the Calculate Volumes automation, which performs the following volume calculations based on the selected Flowcell Type:
   - Calculates Bulk Pool Volume (µl) value.
   - Calculates the Per Sample Volume (µl) value to be added to the pool.
   - Calculates the Total Sample Volume (µl) value.
   - If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) value.
   - Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
   - Generates the Calculation File (*csv) and attaches it to the step. This file contains volume information about the samples and RSB buffer to add to the pool. Select the file to download it, then open it in Excel.
7. Select Next Steps. This selection triggers the Set Next Step automation. This automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol—Dilute, Denature, and ExAmp (NovaSeq 6000 v2.1.
   On the Assign Next Steps screen, the next step is already set to Dilute, Denature, and ExAmp (NovaSeq 6000 v2.1).
8. Select Finish Step.
   At the end of this step, the pool of samples automatically advances to the Dilute, Denature, and ExAmp (NovaSeq 6000 v2.1) step.

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Step 2: Dilute, Denature, and ExAmp (NovaSeq 6000 v2.1)

In this step, pooled samples are denatured and diluted by the addition of DPX, NaOH, Tris-HCl, and RSB. Manually create working pools based on the number of lanes you want to sequence.

Step input: Bulk pool

Step output: Working pool - variable number, choose how many working pools to create per bulk pool

The following automations are configured on the step:

- Calculate Volumes
- Set Next Step
- Validate Inputs Flowcell Type
- Dilute, Denature, and ExAmp (NovaSeq 6000 v2.) automation configuration—Clarity LIMS v4.2
- Dilute, Denature, and ExAmp (NovaSeq 6000 v2.1) automation configuration—Clarity LIMS v5

These automations are described in the order in which they are triggered at run time.

Validate Inputs Flowcell Type

Automatically triggered at the beginning of the step, this automation invokes the `validate_flowcell_for_input_pools` script. This script does the following actions:

- Checks the inputs to the step and validates that the Flowcell Type field has been set to a valid value (SP, S1, S2, or S4). The automation also checks that all inputs have the same value.
- Validates that the number of outputs matches the number of lanes on the selected flow cell type. If validation fails, an error message state that the number of working pools does not match the number of lanes available on the flow cell.

```
bash -l -c "/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/novaseq/novaseq-extensions.jar script:validate_
flowcell_for_input_pools -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1}"
```

Calculate Volumes

Automatically triggered on entry to of the Record Details screen, this automation sets the value of the following fields, based on the Flowcell Type:

- Bp Aliquot Volume (µl)
- NaOH Volume (µl)
- Tris-HCl Volume (µl)
- DPX1 Volume (µl)
- DPX2 Volume (µl)
- DPX3 Volume (µl)

- Mastermix per Lane (µl)

```
if (input.::Flowcell Type:: == ::S1::) {
output.::bp Aliquot Volume (µl):: = 18;
output.::NaOH Volume (µl):: = 4.50;
output.::Tris-HCl Volume (µl):: = 4.50;
step.::DPX1 Volume (µl):: = 126;
step.::DPX2 Volume (µl):: = 18;
step.::DPX3 Volume (µl):: = 66;
output.::Mastermix per Lane (µl):: = 63
};
if (input.::Flowcell Type:: == ::S2::) {
output.::bp Aliquot Volume (µl):: = 22;
output.::NaOH Volume (µl):: = 5.50;
output.::Tris-HCl Volume (µl):: = 5.50;
step.::DPX1 Volume (µl):: = 126;
step.::DPX2 Volume (µl):: = 18;
step.::DPX3 Volume (µl):: = 66;
output.::Mastermix per Lane (µl):: = 77
};
if (input.::Flowcell Type:: == ::S4::) {
output.::bp Aliquot Volume (µl):: = 30;
output.::NaOH Volume (µl):: = 7.50;
output.::Tris-HCl Volume (µl):: = 7.50;
step.::DPX1 Volume (µl):: = 315;
step.::DPX2 Volume (µl):: = 45;
step.::DPX3 Volume (µl):: = 165;
output.::Mastermix per Lane (µl):: = 105
};
```

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs:

```
output.::Flowcell Type::= input.::Flowcell Type::;
output.::Loading Workflow Type::= input.::Loading Workflow Type::;
```

- Uses the `NovaSeq_Xp_Working_Pool.csv` and `NovaSeq_Xp_Working_Pool2.csv` template files to generate a single CSV file containing information about the DPX volume and the volume of bp Aliquot, Mastermix, NaOH, and Tris-HCl to add per working pool. The generated file is available for download on the Step Setup screen of the following step—Load to Flowcell (NovaSeq 6000 v2.0).

Set Next Step

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Automatically triggered on exit of the Record Details screen, this automation:

- Copies the Flowcell Type values from the step inputs to the step outputs.
- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ; nextStep = ::ADVANCE::
```

Step UDFs/Custom Fields

There are three fields defined on the Dilute, Denature, and ExAmp (NovaSeq 6000 v2.1) step. These field values are set by a script and are not editable while running the step.

The fields are shown in the Clarity LIMS v5 Configuration area (Custom Fields > Master Step Fields tab). See Table 12 for Clarity LIMS v4 and Clarity LIMS v5 field configuration details.

Table 289 Table 12: Dilute, Denature, and ExAmp (NovaSeq 6000 v2.1) Step UDF/Custom Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
DPX1 Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0
DPX2 Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0
DPX3 Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0

Global UDFs/Custom Fields

Table 13 lists the global UDFs/custom fields that are configured to display on the Dilute, Denature, and ExAmp (NovaSeq 6000 v2.1) step.

Table 290 Table 13: Global UDF/Custom Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Configured on Analyte in Clarity LIMS v4 and Derived Sample in Clarity LIMS v5:			
Bp Aliquot Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Flowcell Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• SP • S1 • S2 • S4
Loading Workflow Type	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
Mastermix per Lane (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0
NaOH Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Tris-HCl Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2

User Interaction

1. On the Queue screen, add the pool to the Ice Bucket and select View Ice Bucket.
2. On the Ice Bucket screen, set the number of derivatives to create (they are placed into the flow cell lanes) and select Begin Work.
   On entry to the Record Details screen, the Calculate Volumes automation is triggered.
   This automation sets the following values based on the selected Flowcell Type (calculation details are provided in the previous Calculate Volumes Automation section):
   - Bp Aliquot Volume (µl)
   - NaOH Volume (µl)
   - Tris-HCl Volume (µl)
   - DPX1 Volume (µl)
   - DPX2 Volume (µl)
   - DPX3 Volume (µl)
   - Mastermix per Lane (µl)

   The automation also generates the Calculation File (*csv) and attaches it to the step. This file contains information about the DPX Mastermix volume and the volume of Mastermix, NaOH, and Tris-HCl to add per working pool. Select the file to download it, then open it in Excel.

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3. On the Record Details screen, the Reagent Lot Tracking section tracks the DPX1, DPX2, DPX3, NaOH, RSB, and Tris-HCl reagent lots. Select from the active lots displayed in each drop-down list.

   > To make reagent lots available for selection in the Reagent Lot Tracking section, configure and activate them. Do this step on the Reagents and Controls screen (Clarity LIMS v4) or the Configuration > Consumables screen (Clarity LIMS v5).

4. In the Step Details area, the DPX1, DPX2, and DPX3 reagent volume values are already populated. (These values are set by a script and are not editable while running the step.)
5. In the Sample Details table, select the pool icon to view details on the working pool composition.
   - The bp Aliquot, Mastermix per lane, NaOH, and Tris-HCl volume values for each working pool are already populated. (These values are set by a script and are not editable while running the step.)
   - The working pool number is appended to the bulk pool name. This feature helps quickly identify which working pools are derived from the same bulk pool.
6. In the Files area, select the Calculation File (*csv) file to download it. Open the file in Excel to view information about the DPX Mastermix volume and the volume of Mastermix, NaOH, and Tris-HCl to add per working pool.
7. Select Next Steps to trigger the Set Next Step automation (described previously).
   On the Assign Next Steps screen, the next step is already set to Load to Flowcell (NovaSeq 6000 v2.1).
8. Select Finish Step.

Step 3: Load to Flowcell (NovaSeq 6000 v2.1)

In this step, scan the flow cell barcode into Clarity LIMS, then manually place the working pools into the lanes of the flow cell that is used in the NovaSeq run.

Step input: Working pool

Step output: Flow cell (output containers: S1 and S2 with 2 lanes, and S4 with 4 lanes)

The following automations are configured on this step:

- Generate Sample Sheet and Run Recipe
- Prepare Files for NovaSeq
- Validate Flowcell Barcode
- Validate Inputs and Selected Container
- Load to Flowcell (NovaSeq 6000 v2.0) automation configuration—Clarity LIMS v4
- Load to Flowcell (NovaSeq 6000 v2.1) automation configuration—Clarity LIMS v5

These automations are described in the order in which they are triggered at run time.

Validate Inputs and Selected Container

Automatically triggered at the beginning of the step, this automation invokes the `validate_flowcell_for_input_pools` and `validateSelectedContainer` scripts. These scripts validate the step inputs and the container selected, as follows.

- Check that the Flowcell Type field has been set to a valid value (S1, S2, S4, or SP) and that all inputs have the same value.
- Check that the number of outputs matches the number of lanes on the selected flow cell type. If validation fails, an error message states that the number of working pools does not match the number of lanes available on the flow cell.

The following table defines the number of samples allowed:

Flowcell Type	Number of Working Pools
SP	2
S1	2
S2	2
S4	4

- Check that the container type selected on entry to the Placement screen matches the value in the Flowcell Type field. If validation fails, an error message displays.

Validate Flowcell Barcode

Automatically triggered on exit of the Placement screen, this automation validates the flow cell barcode scanned into Clarity LIMS, using the following logic:

```
if (input.::Flowcell Type:: == ::S1:: && !output.container.name.matches( ::[A-Za-z0-9]{5}DRXX:: ) ) {   fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: )
} ;
if (input.::Flowcell Type:: == ::S2:: && !output.container.name.matches( ::[A-Za-z0-9]{5}DMXX:: ) ) {   fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: )
   } ;
if (input.::Flowcell Type:: == ::S4:: && !output.container.name.matches( ::[A-Za-z0-9]{5}DSXX:: ) ) {   fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: )
 } ;
```

Generate Sample Sheet and Run Recipe

Automatically triggered when a button on the Record Details screen is selected, this automation:

- Copies the Flowcell Type from the step input to the Run Mode field (hidden):

```
step.::Run Mode:: = input.::Flowcell Type::
```


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- Validates the parameters entered on the Record Details screen:
  - Experiment Name can only contain alphanumeric, dash, or underscore characters. Spaces are not permitted.
  - When Workflow Type is No Index, Index Read 1 must be zero. For any other Workflow Type, Index Read 1 must be greater than zero.

```
if(!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) {
fail(::Experiment Name contains prohibited characters. Allowed characters are:
a-z, A-Z, 0-9, -, and _::)
};
if(step.::Workflow Type::== ::No Index::) {
if(step.::Index Read 1::!= 0) {
   fail(::Index Read 1 must be 0 if the Workflow Type is No Index.::)
  }
} else{
if(step.::Index Read 1::== 0) {
fail(::Index Read 1 must be greater than 0 if the Workflow Type is ::+
step.::Workflow Type::+ ::.::)
   }
};
```

  - When Paired End is True, Index Read 1 and Index Read 2 must be greater than zero.
  - When Paired End = False, sets Read 2 Cycles to 0.

```
step.::Read 2 Cycles:: = step.::Paired End::.toBoolean() ? step.::Read 1
Cycles:: : 0;
```

  - Determines Index Read 2 based on the Workflow Type and Index Read 1. When Workflow Type is No Index or Single Index, Index Read 2 is set to zero.
  - When Workflow Type is Dual Index or Custom, Index Read 2 is set to the same value as Index Read 1.

```
if (step.::Workflow Type:: == ::No Index:: || step.::Workflow Type:: == ::Single
Index::) { step.::Index Read 2:: = 0 } else { step.::Index Read 2:: =
step.::Index Read 1:: }
```

  - Copies the Flowcell Type value from the step inputs to the step outputs:

```
output.::Flowcell Type::= input.::Flowcell Type::;
```

  - Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol—AUTOMATED - Run (NovaSeq 6000) v2.0.

```
nextStep = ::ADVANCE::
```

  - Generates the sample sheet and the run recipe file and attaches them to the step. For details on how the files are generated, and their contents, see the *Sample Sheet and Run Recipe File Generation* section.

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Prepare Files for NovaSeq

Automatically triggered on exit of the Record Details screen, this automation invokes the `copy_attachments_to_network_folder` script, which is included in the `novaseq-remote-extensions.jar` file. This script places the generated sample sheet and run recipe (*json file) into the appropriate folder on the NAS, where the instrument uses them to start the run.

For more information, see the *Sample Sheet and Run Recipe File Generation* section.

Step UDFs/Custom Fields

There are 15 fields defined on the Load to Flowcell (NovaSeq 6000 v2.1) step.

These fields are shown in the Clarity LIMS v5 Configuration area (Custom Fields > Master Step Fields tab). See Table 14 for Clarity LIMS v4 and Clarity LIMS v5 field configuration details.

Table 291 Table 14: Load to Flowcell (NovaSeq 6000 v2.1) Step UDF/Custom Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
BaseSpace Sequence Hub Configuration	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• Not Used • Run Monitoring Only • Run Monitoring and Storage
Experiment Name	Single-line Text (v4) Text (v5)	• Required: Yes • Read Only: No	Not applicable
Index Read 1	Numeric (v4) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 0 • 6 • 8 • Range From: 0 to 20 • Decimal Places Displayed: 0
Index Read 2	Numeric (v4) Numeric (v5)	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Library Tube Barcode	Numeric (v4) Numeric (v5)	• Required: Yes • Read Only: No	• Decimal Places Displayed: 0
Paired End	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• True • False
Read 1 Cycles	Numeric (v4) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 251 • 151 • 101 • 51 • Range From: 1 to 251 • Decimal Places Displayed: 0
Read 2 Cycles	Numeric (v4) Numeric Dropdown (v5)	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0
Reverse Complement Workflow	Check Box (v4) Toggle Switch (v5)	• Required: No • Read Only: Yes	• Default: Yes
Run Mode (not displayed in user interface)	Single-line Text (v4) Text Dropdown (v5)	• Required: No • Read Only: Yes • Custom Entries: No	• S1 • S2 • S4 • SP
Sample Sheet Path	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
UMI—Read 1 Length	Numeric (v4) Numeric (v5)	• Required: No • Read Only: No	• Range From: 1 • Decimal Places Displayed: 0
UMI—Read 1 Start From Cycle	Numeric (v4) Numeric (v5)	• Required: No • Read Only: No	• Range From: 1 • Decimal Places Displayed: 0
UMI—Read 2 Length	Numeric (v4) Numeric (v5)	• Required: No • Read Only: No	• Range From: 1 • Decimal Places Displayed: 0
UMI—Read 2 Start From Cycle	Numeric (v4) Numeric (v5)	• Required: No • Read Only: No	• Range From: 1 • Decimal Places Displayed: 0
Use Custom Index Read 1 Primer (For Clarity LIMS v5.0.5 and later, see Configuration Update documentation.)	Check Box (v4) Toggle Switch (v5)	• Required: No • Read Only: No	• Default: No
Use Custom Read 1 Primer (For Clarity LIMS v5.0.5 and later, see Configuration Update documentation.)	Check Box (v4) Toggle Switch (v5)	• Required: No • Read Only: No	• Default: No
Use Custom Read 2 Primer (For Clarity LIMS v5.0.5 and later, see Configuration Update documentation.)	Check Box (v4) Toggle Switch (v5)	• Required: No • Read Only: No	• Default: No
Workflow	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	• GenerateFASTQ

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Workflow Type	Single-line Text (v4.2) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• No Index • Single Index • Dual Index

Global UDFs/Custom Fields

Table 15 lists the global UDFs / custom fields that are configured to display on the Load to Flowcell (NovaSeq 6000 v2.1) step.

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Configured on Analyte in Clarity LIMS v4 and Derived Sample in Clarity LIMS v5:			
Flowcell Type	• Single-line Text (v4.2) • Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• S1 • S2 • S4 • SP

User Interaction

1. On the Ice Bucket screen, select the appropriate flow cell type from the Destination Container drop-down list in the Container Options panel.
2. Select Begin Work.
3. On the Placement screen:
   a. Drag the pools from the left of the screen over into the Placed Samples area on the right.
   b. Scan or type the barcode of the flow cell into the flow cell field.
   c. Select Record Details.
   On exit of the Placement screen, the Validate Flowcell Barcode automation validates the container barcode (described previously).
4. The fields displayed on the Record Details screen are used to generate the sample sheet and run recipe files. Some of these fields are autopopulated and some must be completed manually (see Table 16 for details).

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Table 292 Table 16: Fields Displayed on Record Details Screen of Load to Flowcell (NovaSeq 6000 v2.1) Step

Field	Value
Experiment Name	Enter the name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.
BaseSpace Sequence Hub Configuration	Select from preset options: Not Used, Run Monitoring Only, or Run Monitoring and Storage.
Workflow	Automatically populated with preset—GenerateFASTQ.
Workflow Type	Select from preset options: No Index, Single Index, Dual Index, or Custom.
Index Read 1	Select from preset option: 0, 6, or 8—or types a value between 0 and 20.
Index Read 2	Select from preset options: 0, 6, 8—or type a value between 0 and 20.
Paired End	Select from preset options: True or False.
Read 1 Cycles	Select from preset options: 251, 151, 101, or 51—or type a value between 1 and 251.
Read 2 Cycles	Select from preset options: 251, 151, 101, or 51—or type a value between 1 and 251.
Use Custom Read 1 Primer	Select if applicable.
Use Custom Read 2 Primer	Select if applicable.
Use Custom Index Read 1 Primer	Select if applicable.
Reverse Compliment Workflow	Select Yes (by default) for v1.5 reagents in NovaSeq Control Software v1.7 and onwards. Select No for v1 reagents.
UMI - Read 1 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 1. Leave blank if not applicable.
UMI - Read 2 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 2. Leave blank if not applicable.
UMI - Read 1 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.
UMI - Read 2 Start From Cycle	Enter the cycle number that Read 2 starts from. Leave blank if not applicable.
Sample Sheet Path	Automatically populated after the sample sheet has been generated.
Library Tube Barcode	Scan the library tube barcode.

5. On the Record Details screen, select Generate Sample Sheet and Run Recipe. This selection triggers the automation script, which does the following actions:
   - Generates the sample sheet and JSON files and attaches them to the placeholders in the Files area of the Record Details screen.
   - Populates the Sample Sheet Path field with the path to the sample sheet file.
6. Select Next Steps to trigger the Prepare Files for NovaSeq automation, which copies the sample sheet and run recipe files to the location specified during installation.
   On the Assign Next Steps screen, the Next Step for all samples is prepopulated with Mark protocol as complete.
7. Select Finish Step.
   In Lab View, the pool of samples is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.1) step.

At this point in the workflow, the user interaction ends. The integration automatically starts the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.1) step and data from the run is parsed back into Clarity LIMS.

Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.1)

This final protocol contains one fully automated step, AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.1).

Step 1: AUTOMATED - NovaSeq Run (NovaSeq 6000) 2.1

Step input: Library tube from NovaSeq Standard protocol or NovaSeq Xp protocol

Step output: Result file / measurement

> Do not start this step. This step is a fully automated and the sequencing service does not update samples correctly if they have been manually started.

In this step, pooled samples are sequenced on the NovaSeq instrument. The run data network folder is monitored by the sequencing service and automation worker to determine when the sequencing run begins and ends.

1. When initiating a run on the NovaSeq instrument, the NovaSeq Control Software (NVCS) looks for the sample sheet and run recipe (*.json file) on the shared network drive. The NVCS copies the sample sheet to the run directory.
   - The NVCS uses the run recipe to start the sequencing run.
2. When the run starts, the instrument software creates a new run folder (named < libraryID >) on the shared network drive and copies the following files into that folder:
   - RunParameters.xml
   - RunInfo.xml
   - LIMS/ <libraryID>.json file (run recipe)

- The Real-Time Analysis v3 (RTA3) software creates the InterOp folder.

3. The sequencing service detects the presence of the RunParameters, RunInfo, and JSON files and starts the AUTOMATED- NovaSeq Run (NovaSeq 6000 v2.1) step in Clarity LIMS.
4. As the run progresses, the InterOp data files are filled in.
5. At the end of the run, the NovaSeq copies the run data files to the InterOp folder and creates the `CopyComplete.txt` file in the run folder.
   This step indicates to the sequencing service that the run has completed and the data files are ready.
6. The sequencing service triggers the Read InterOp Metrics automation. This automation reads the files, records the parsed metrics into Clarity LIMS, and finally completes the step in Clarity LIMS.
   The following automations are configured on the step:
   - Parse Run Parameters and Generate Link to Run Folder
   - Read InterOp Metrics
   - Set Next Steps
   - Update Lane Number
   - AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.1) automation configuration—Clarity LIMS v4
   - AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.1) automation configuration—Clarity LIMS v5

These automations are described in the order in which they are triggered at run time.

Parse Run Parameters and Generate Link to Run Folder

Automatically triggered on entry to the Record Details screen, this automation does the following actions:

- Parses the `RunParameters.xml` file attached on the Step Setup screen.
- Populates the step UDFs/Custom Fields with extracted values.
- If there is an instrument in Clarity LIMS that matches the instrument name in the file (InstrumentName field), the script associates this instrument with the run.
- Generates and attaches a link to the run folder using the run output folder parsed from `RunParameters.xml`.

Rules and Constraints

- If there is no InstrumentName value in the file, the script continues, but it logs a warning.
- If there is an InstrumentName value in the file and multiple instruments with the same name exist in Clarity LIMS, the script fails and logs an error.
- If the `RunParameters.xml` file is not attached to the step during step setup, the script fails.

- Every `RunParameters.xml` field that has an equivalent step UDF/custom field in Clarity LIMS is required—except for InstrumentName. If a field is not present in the `RunParameters.xml` file, Clarity LIMS logs an error to this effect.

Step UDFs/Custom Fields

There are 23 fields defined on the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.1) step. The fields are shown in the Clarity LIMS v5 Configuration area (Custom Fields > Master Step Fields tab).

Table 17 shows how the parsed `RunParameters.xml` fields map to step UDFs/custom fields configured on the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.1) step in Clarity LIMS.

Table 293 Table 17: Fields Parsed by parse_novaseq_run_parameters script

RunParameters.xml Field	Step UDF/Custom Field Name
FlowCellSerialBarcode	Flow Cell ID
FlowCellPartNumber	Flow Cell Part Number
FlowCellLotNumber	Flow Cell Lot Number
FlowCellExpirationdate	Flow Cell Expiration Date
FlowCellMode	Flow Cell Mode
RunId	Run ID
ClusterSerialBarcode	PE Serial Barcode
ClusterPartNumber	PE Part Number
ClusterLotNumber	PE Lot Number
ClusterExpirationdate	PE Expiration Date
ClusterCycleKit	PE Cycle Kit
SbsSerialBarcode	SBS Serial Barcode
SbsPartNumber	SBS Part Number
SbsLotNumber	SBS Lot Number
SbsExpirationdate	SBS Expiration Date
SbsCycleKit	SBS Cycle Kit
BufferSerialBarcode	Buffer Serial Barcode
BufferPartNumber	Buffer Part Number
BufferLotNumber	Buffer Lot Number
BufferExpirationdate	Buffer Expiration
OutputRunFolder	Output Folder

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RunParameters.xml Field	Step UDF/Custom Field Name
WorkflowType	Loading Workflow Type

Read InterOp Metrics

Automatically triggered when the sequencing service detects the `CopyComplete.txt` file has been created, this automation parses the InterOp data generated by the run.

- Read summary metrics are recorded on the library pool. These values are aggregated across all lanes. Some values—for example Yield PF (Gb)—are summed, while others are averaged.
- Per lane metrics are recorded on the measurement outputs.

The `read_interop_metrics` script loads data from the InterOp files and records summary metrics into derived sample UDFs/custom fields in Clarity LIMS. If any field is not configured or cannot be written to, the script fails.

Global UDFs/Custom Fields

The global UDFs/custom fields that are configured to support the parsing of key metrics back into Clarity LIMS are as follows.

- Yield PF (Gb) R1
- Yield PF (Gb) R2
- Reads PF (M) R1
- Reads PF (M) R2
- Cluster Density (K/mm^2) R1
- Cluster Density (K/mm^2) R2
- %PF R1
- %PF R2
- % Bases >=Q30 R1
- % Bases >=Q30 R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- % Aligned R1

- % Aligned R
- % Error Rate R1
- % Error Rate R2

Set Next Steps

Automatically triggered on exit of the Record Details screen, this automation sets the next step to ADVANCE and the samples complete the protocol.

```
nextStep = ::ADVANCE::
```

Update Lane Number

Automatically triggered on exit of the Step Setup screen, this automation updates the lane number so that Read InterOp Metrics automation script can populate lane metric to the correct lane.

Do not disable Update Lane Number automation as it causes run metric parsing to work improperly.

Components Installed with This Integration

The following sections describe the various components that are installed by default as part of this integration. These components include files, database properties, reagent categories/label groups, reagent kits, and containers. Information on installed workflows, protocols, steps, and automation points is provided in the previous *Workflows, Protocols, and Steps* section.

The Illumina NovaSeq 6000 Integration v2.3 is distributed as two RPM packages:

- BaseSpaceLIMS-novaseq-extensions
- BaseSpaceLIMS-novaseq-sequencing-service

The RPM packages install the components listed in the following table.

The NovaSeq 6000 Integration requires installation of NGS Extensions Package v5.20 or later.

- If the NGS Extensions Package is not already installed, or if a version earlier than v5.20 is installed, v5.20 is installed by default with the NovaSeq integration. See Release Notes for details.
- If NGS Extensions Package v5.20 is already installed, upgrade is not forced or required.
- Secret Util Package is required for the NGS Extensions Package v5.20.
- If the Secret Util Package is not already installed, then Secret Util package is installed along with the installation of NGS Extension Package v5.20.
- If the Secret Util Package is already installed, upgrade is not forced or required.

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Table 294 Table 18: Illumina NovaSeq 6000 Integration RPM Components

Files Installed	Location and Description
configure_extensions_novaseq.sh	/opt/gls/clarity/config/ Script that imports Clarity LIMS configuration into the application database and updates property values.
novaseq-extensions.jar	/opt/gls/clarity/extensions/novaseq Jar file containing API-based Clarity LIMS extensions used throughout the protocols.
novaseq-sequencing.jar	/opt/gls/clarity/extensions/novaseq/SequencingService Sequencing service jar file that captures the run results.
novaseq-remote-extensions.jar	/opt/gls/clarity/extensions/novaseq Jar file containing scripts that require access to the run folders on the NAS.
InterOp libraries	/opt/gls/clarity/extensions/novaseq/lib Illumina shared library for parsing InterOp data files.
• NovaSeq_Standard_Bulk_Pool1.csv • NovaSeq_Standard_Bulk_Pool2.csv • NovaSeq_Standard_Bulk_Pool3.csv • NovaSeq_Xp_Bulk_Pool.csv • NovaSeq_Xp_Bulk_Pool2.csv • NovaSeq_Xp_Working_Pool.csv • NovaSeq_Xp_Working_Pool2.csv	/opt/gls/clarity/extensions/novaseq/Templates Template files used for file generation throughout the protocols.

Database Properties

Table 19 lists the database properties installed with the Illumina NovaSeq 6000 Integration Package.

Table 295 Table 19: Database Properties Installed

Property	Description
novaseq.runSetupFolder	The directory where the run recipe files are written.

Property	Description
novaseq.sampleSheetPathPrefixSearch.SUFFIX	The directory where the instrument control software or analysis software looks for sample sheets, typically a Windows path to a network drive.
novaseq.sampleSheetPathPrefixReplace.SUFFIX	The directory where the sample sheets are written.
novaseq.sampleSheetPathPrefixSearchReplaceSuffixes*	Search and replace entries for transforming Windows to Linux network paths. This property must be set to the single SUFFIX used in the two previous properties.
novaseq.seqservice.netPathPrefixSearch.SUFFIX	The search prefix used to map the OutputRunFolder field in `RunParameters.xml` to the corresponding Linux directory.
novaseq.seqservice.netPathPrefixReplace.SUFFIX	The directory where the sequencing service looks for run folders.
novaseq.seqservice.netPathPrefixSearchReplaceSuffixes*	Search and replace entries for transforming Windows to Linux network paths. This property must be set to the single SUFFIX used in the two properties above.

*This version of the integration only supports a single active search-replace pair of each type:sampleSheetPathPrefixSearchReplaceSuffixes and netPathPrefixSearchReplaceSuffixesmust each contain a single number, rather than a comma-separated list of numbers.

Reagent Categories/Label Groups

- TruSeq HT Adapters v2 (D7-D5)

Reagent Kits

- DPX1
- DPX2
- DPX3
- Resuspension Buffer (RSB)
- NaOH

- Tris HCl

Container Types

- Library Tube

Instrument Integration

The following are instructions for configuring the NovaSeq instrument for the Clarity LIMS integration:

1. Launch NovaSeq Control Software.
2. On the home screen, select the menu (top left) and select Settings.
3. Select File-Based and enter the network folder location that NovaSeq Control Software retrieves the JSON recipe from.
4. Next enter the network output folder location.
5. Select Save.

For more information, refer to *NovaSeq Sequencing System Guide*.

Routing Script Requirements

The requirements for the routing script functionality are as follows.

- On the steps that use the routing script—Define Run Format (NovaSeq 6000 v2.1) and Dilute and Denature (NovaSeq 6000 v2.1)—the Next Step for all samples must be set to Remove from workflow. This value is set by a script. Do not change this value in the Assign Next Steps screen.
- Clarity LIMS v5: In the protocol configuration screen, the following settings are required and must not be changed. In the Next Steps section for the last step in the protocol, the method of assigning the next step must be set to Automatic.
- Clarity LIMS v4.2: In the protocol configuration area, for all protocol steps on which the Routing Script automation is configured, the following settings are required and must not be changed:
  - On the Next Steps configuration tab, the Next Steps are set by a script toggle switch must be set to Yes.

Sample Sheet and Run Recipe File Generation

Sample sheet and run recipe file generation occurs on the step before the run—Dilute and Denature (NovaSeq 6000 v2.1) in the NovaSeq Standard protocol or Load to Flowcell (NovaSeq 6000 v2.1) in the NovaSeq Xp protocol. This step is where samples are placed on the library tube or flow cell that is loaded in the NovaSeq instrument.

In the out-of-the-box configuration, the Generate Sample Sheet and Run Recipe automation generates the following:

- One CSV format sample sheet file for use bcl2fastq v2.20 downstream analysis
- One JSON format run recipe file

The Prepare Files for NovaSeq automation places the generated files on the NAS where the instrument uses them to start the run.

How It Works

- The Prepare Files for NovaSeq automation command line invokes the `copy_attachments_to_network_folder` script `in novaseq-remote-extensions.jar`. The script can be used on a remote AI node/automation worker, in case the Clarity LIMS server does not have direct access to the NAS.
- The output folder for the run recipe file is found in the `novaseq.runSetupFolder` property.
- The output folder for the sample sheet is determined by using the `novaseq.sampleSheetPathSearchReplaceSuffixes` property. This property must contain exactly one suffix (not a comma-separated list) to derive a full property name based on `novaseq.sampleSheetPathPrefixReplace`.

Sample Sheet Contents

The contents of the sample sheet are controlled by the following fields configured on the Dilute and Denature (NovaSeq 6000 v2.1) and Load to Flowcell (NovaSeq 6000 v2.1) steps:

- Experiment Name
- Read 1 Cycles
- Read 2 Cycles
- Workflow
- Reverse Complement Workflow
- Read1UMILength
- Read2UMILength
- Read1StartFromCycle
- Read2StartFromCycle

See the *Bcl2fastq2 Sample Sheet Generation* article for detailed information on the following:

- Sample sheet generation script parameters and usage
- Sample sheet data and configuration options
- Enabling unique FASTQ file names per sequencing run

Run Recipe Contents

The contents of the run recipe are controlled by the following fields. These fields are configured on the Dilute and Denature (NovaSeq 6000 v2.1) and Load to Flowcell (NovaSeq 6000 v2.1) steps.

- Experiment Name
- Run Mode
- Workflow Type

- Sample Loading Type (populated based on Workflow field)
- Paired End
- Read 1
- Read 2
- Index Read 1
- Index Read 2
- Sample Sheet Path
- BaseSpace Configuration
- Use Custom Read 1 Primer
- Use Custom Read 2 Primer
- Use Custom Index Read 1 Primer

For Dilute and Denature (NovaSeq 6000 v2.1) step field configuration details, see Table 7. To see how these fields are populated on the Record Details screen at run time, see Table 9.

For Load to Flowcell (NovaSeq 6000 v2.1) step field configuration details, see Table 14. To see how these fields are populated on the Record Details screen at run time, see Table 16.

The following fields are also included in the run recipe:

- Library Tube Barcode (librarytube_ID in JSON file)—For NovaSeq Standard workflow, the library tube barcode is derived from the container name entered or scanned on the Placement screen. For NovaSeq Xp workflow, the library tube barcode is derived from the Library Tube Barcode UDF in Load To Flowcell (NovaSeq 6000 v2.1) step.
- Flowcell Barcode (flowcell_ID in JSON file)—For NovaSeq Standard workflow, the library tube barcode is not output to JSON recipe file. For NovaSeq Xp workflow, the flow cell barcode is derived from the container name entered or scanned on the Placement screen.
- Rehyb—preset to False.
- Output Folder (output_folder in JSON file)—Derived from the system property novaseq.seqservice.netPathPrefixSearch.
- Attachment—Derived from the system property novaseq.sampleSheetPathPrefixSearch with the name of the sample sheet added to the end of the path.
- Use BaseSpace (use_basespace in JSON file)—Set to False if the BaseSpace Sequence Hub Configuration step UDF/custom field value is Not Used, and true otherwise.

Rules and Constraints

- The sample sheet and run recipe files are mandatory inputs for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.1) step. For the automated run to start successfully, select the Generate Sample Sheet and Run Recipe button to generate these files.

- The output folders must exist on the system where the script runs (can be a remote AI node/automation worker).
- The output folders must be writable by the glsai user.

Support for Container Types

This integration supports the following container types:

- Library tube with barcode provided in the format:
  - [A-Za-z]{2}[0-9]{7}-[A-Za-z]{3}
  - Example: NV1234567-LIB
- Flow cell with barcode provided in one of the following formats:
  - For SP and S1: [A-Za-z0-9]{5}
  - DRX[XY2357]
  - For S2: [A-Za-z0-9]{5}
  - DMX[XY2357]
  - For S4: [A-Za-z0-9]{5}
  - DSX[XY2357]
  - Example: S2 Flowcell barcode

Example: H1234DMXX

Rules and Constraints

- The workflow configuration contains several validation checks. To make sure that the calculations work properly, it is important that you do not disable any of this validation logic. The validation checks the following information:
  - Which samples, and how many, can enter each step together.
  - Which samples, and how many, can be pooled together.
- Reagent labels (indexes) must be unique.
- The library tube ID must be unique. There should not be multiple library tube containers in the system with the same name.
- Only controls are permitted as unindexed samples. All other unindexed samples and pools are not permitted.
- For sample sheet generation constraints, refer to Bcl2fastq2 Sample Sheet Generation.
- Do not manually start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.1) step. This step is fully automated. If the samples have been manually started, the sequencing service does not update them correctly.
- The output folder must be readable by the Clarity LIMS glsjboss account on the server.

NovaSeq 6000 Integration v2.3 Validation and Troubleshooting

This guide explains how to validate the installation of the Illumina NovaSeq 6000 Integration Package v2.3.

The validation process involves the following actions:

- Running samples through the NovaSeq Validation Library Prep (NovaSeq 6000 v2.1) workflow. The workflow contains a single-step protocol that models the library prep required to produce normalized libraries. At the end of the step, the normalized libraries are automatically advanced to the NovaSeq 6000 v2.1 workflow.
- Running normalized libraries through the NovaSeq 6000 v2.1 workflow. This process validates the following details:
  - Successful routing of samples from the Run Format (NovaSeq 6000 v2.1) step to the NovaSeq Standard (NovaSeq 6000 v2.1) or NovaSeq Xp (NovaSeq 6000 v2.1) step.
  - Automated generation of a sample sheet for use with bcl2fastq2 v2.20 analysis software.
  - Automated generation of a run recipe file (JSON file) with the library tube or flow cell barcode as the name (eg, NV1234567-LIB.json or H1234DRXX.json). This file is automatically uploaded to the sequencing instrument and used to set up and initiate the run.
  - Automated tracking of the NovaSeq sequencing run and parsing of run statistics (per run per lane) into the LIMS.

Before executing the validation steps, make sure that you have installed the Illumina NovaSeq 6000 Integration Package v2.3.0 and have imported the default Clarity LIMS configuration.

Validation steps

Activate workflow, create project, add and assign samples

The following steps set up Clarity LIMS in preparation for running samples through the NovaSeq Validation Library Prep (NovaSeq 6000 v2.1) and NovaSeq 6000 v2.1 workflows.

1. Activate the workflow

1. In the Clarity LIMS configuration area, activate the NovaSeq Validation Library Prep (NovaSeq 6000 v2.1) and NovaSeq 6000 v2.1 workflows.

2. Create a project, add samples

1. On the Projects and Samples screen, create a project and add samples to it.
2. Assign your samples to the NovaSeq Validation Library Prep (NovaSeq 6000 v2.1) workflow.

Library Prep Protocol: NovaSeq Validation Library Prep (NovaSeq 6000 v2.1)

This single-step protocol models the library prep required to produce normalized libraries that are ready for the NovaSeq 6000 v2.1 workflow.

Run NovaSeq Validation Library Prep (NovaSeq 6000 v2.1) step

1. In Lab View, locate the NovaSeq Validation Library Prep (NovaSeq 6000 v2.1) protocol. The samples are queued for the NovaSeq Validation Library Prep (NovaSeq 6000 v2.1) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Placement screen, scan or enter the barcode of the destination container into the Container field of the Placed Samples area.
   a. Select the samples from the Samples to be Placed area on the left, then drag them over to the container wells on the right.
   b. Select Add Labels.
5. On the Add Labels screen, select labels from the Labels to Add list and drag them to the container on the right to assign them to placed samples.
   The TruSeq HT Adapters v2 (D7-D5) label group is the only label group configured in the out-of-the-box integration and is the default in this step.
   a. Select Record Details.
6. On the Record Details screen in the Sample Details table, enter values for Normalized Molarity.
   a. Type values individually or use the copy/paste functionality to enter multiple values from a spreadsheet.

      If you are working with many samples, use the copy/paste functionality to enter multiple values from a spreadsheet.

      1. Select the Copy button to copy the sample information from Clarity LIMS to the clipboard.
      2. Open a blank Excel document and paste the contents of the clipboard into the spreadsheet.
      3. In the spreadsheet, enter the Normalized Molarity values. Select the entire spreadsheet, including the headers (Ctrl + A), and copy (Ctrl + C) the information to the clipboard.
      4. In Clarity LIMS, select the Paste button. Paste the contents of the clipboard (Ctrl + V) into the Update Table window, then select Update to paste into the Sample Details table.

   b. The Sequencer Type field indicates the instrument to be used for the sequencing run. In this integration, the value of this field is set to NovaSeq automatically.
5. Select Next Steps.

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This selection triggers the Next Steps automation, which sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step successfully.

6. On the Assign Next Steps screen in the Samples table, the Next Step for all samples is prepopulated with Remove from workflow.

   Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.

7. Select Finish Step.
   On exit from the step, the Routing Script automation is triggered. This automation assigns samples to the first step of the NovaSeq 6000 v2.1 workflow—Define Run Format (NovaSeq 6000 v2.1). This step is the only one in Protocol 1: Run Format (NovaSeq 6000 v2.1).

Protocol 1: Run Format (NovaSeq 6000 v2.1)

This protocol includes a single step—Define Run Format (NovaSeq 6000 v2.1). The step allows for the assignment of per sample values for Loading Workflow Type, Normalized Molarity, Flowcell Type, and Final Loading Concentration (pM). At the end of the step, samples are routed to the NovaSeq Standard or NovaSeq Xp protocol, according to the selected Loading Workflow Type.

Run Define Run Format (NovaSeq 6000 v2.1) Step

1. In Lab View, locate the Run Format (NovaSeq 6000 v2.1) protocol. The samples are queued for the Define Run Format (NovaSeq 6000 v2.1) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Record Details screen, specify the Sample Details.
   - Loading Workflow Type—Select NovaSeq Standard or NovaSeq Xp from the drop-down list.
   - Normalized Molarity—Enter values or use values copied from the previous step.
5. Populate the following fields (values can vary across samples):
   - Flowcell Type—Select SP, S1, S2, or S4.
   - Final Loading Concentration (pM)—Select from the two preset options—225 (for PCR-free workflows) or 400 (for Nano workflows)—or enter a different value.
6. Select Next Steps to trigger the Next Steps automation, which does the following action:
   - Sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step successfully.
   - Calculates the Minimum Molarity.

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- Checks Normalized Molarity value. For samples with no Normalized Molarity value (ie, an empty value, not including 0), the automation generates an error message stating that the field cannot be empty.
- Compares the Normalized Molarity value of each sample with the Minimum Molarity value.

7. On the Assign Next Steps screen, review the Sample Details table and make sure that the Next Step for all samples is prepopulated with Remove from workflow. Next Step must be set to Remove from workflow, regardless of the Loading Workflow Type.

   If the Next Step value is not set to Remove from workflow, the routing script automation cannot advance to the next step.

8. Review the Warning field entries for the samples. If the field indicates that the Normalized Molarity is too low, continue with one of the following options:
   - Return to the Record Details screen and adjust the Normalized Molarity value so that it equals or exceeds the Minimum Molarity value. Set the Loading Workflow Type to NovaSeq Standard or NovaSeq Xp, as applicable.
   - Alternatively, complete the protocol without correcting the Normalized Molarity value. In this case, the samples in question are removed from the Clarity LIMS workflow.
9. Select Finish Step. The Routing Script automation is triggered:
   - Samples with a Loading Workflow Type of Remove from workflow (ie, where Normalized Molarity value is lower than the Minimum Molarity) are removed from the Clarity LIMS workflow.
   - Samples with a Loading Workflow Type of NovaSeq Standard are routed to the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.1) step. This step is the first of two steps in Protocol 2: NovaSeq Standard (NovaSeq 6000 v2.1).
   - Samples with a Loading Workflow Type of NovaSeq Xp are routed to the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.1) step. This step is the first of three steps in Protocol 3: NovaSeq Xp (NovaSeq 6000 v2.1).

Protocol 2: NovaSeq Standard (NovaSeq 6000 v2.1)

In this protocol, samples are pooled and added to the library tube in preparation for the NovaSeq run. The protocol contains two steps:

1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.1)
2. Dilute and Denature (NovaSeq 6000 v2.1)

Step 1: Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.1)

1. In Lab View, locate the NovaSeq Standard (NovaSeq 6000 v2.1) protocol. The samples are queued for the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.1) step.
2. On the Queue screen, add samples of the same flow cell type to the Ice Bucket and select Begin Work.

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3. On the Pooling screen, create a pool by dragging samples into the Pool Creator. Only one pool must be created.
   a. Type a name for the pool or accept the default name (Pool #1).
   b. Select Record Details.
4. On exit from the Pooling screen, the Validate Inputs Flowcell Type and Single Pool is triggered. The automation checks the following information:
   - All samples in the pool have been assigned the same Flowcell Type.
   - Only one pool has been created.
5. On the Record Details screen in the Step Details area, complete the two required fields and one optional field as needed:
   - Number of Flowcells to Sequence—The value entered in this field is used in volume calculations to make sure that volumes are sufficient for the number of times the pool is sequenced.
   - Minimum Per Sample Volume (µl)—The value in this field is used to calculate how much of each sample is included in the pool. The field is prepopulated with the configured default value (5 µl), but the value can be edited if necessary.
   - % PhiX (2.5 nM) Spike-In—The value in this field is used to calculate the volume of PhiX v3 control to be included in the pool for the given percentage of spike-in. The field is optional and can be edited if necessary.

     Assuming the default Minimum Per Sample Volume (µl) value of 5, for a given batch:
     - If the smallest Per Sample Volume (µl) value is less than 5, the LIMS automatically assigns a value of 5 to the sample's Adjusted Per Sample Volume (µl) field.
     - Clarity LIMS then adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.
6. In the Sample Details table, select the pool icon to view details on the pool composition.
7. Select Calculate Volumes to trigger the Calculate Volumes automation, which performs volume calculations based on the selected Flowcell Type:
   - Calculates Bulk Pool Volume (µl) value.
   - Calculates the Per Sample Volume (µl) value to be added to the pool.
   - Calculates PhiX Volume (µl) value if % PhiX (2.5 nM) Spike-In has value.
   - Calculates the Total Sample Volume (µl) value.
   - If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) value.
   - Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.

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- Populates the Volume of Pool to Denature (µl), NaOH Volume (µl) and Tris-HCl Volume (µl) columns of the Sample Details table. (Values are set by a script and are not editable.)
- The automation also generates the Calculation File (*csv) and attaches it to the step. This file contains volume information about the pool and the individual samples it contains. Select the file to download it, then open it in Excel.

8. Select Next Steps to trigger the Set Next Step automation. This automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol—Dilute and Denature (NovaSeq 6000 v2.1).
   On the Assign Next Steps screen, the next step for samples is already set to the next step in the workflow—Dilute and Denature (NovaSeq 6000 v2.1).
9. Select Finish Step.

At the end of this step, the pool of samples automatically advances to the Dilute and Denature (NovaSeq 6000 v2.1) step.

Step 2: Dilute and Denature (NovaSeq 6000 v2.1)

1. In Lab View, locate the NovaSeq Standard (NovaSeq 6000 v2.1) protocol. The pool of samples is queued for the Dilute and Denature (NovaSeq 6000 v2.1) step.
2. Add the samples to the Ice Bucket and select Begin Work.
   At the beginning of the step, the Validate Single Input automation is triggered. This automation checks that there is only one container input to the step.
3. On the Placement screen, drag the pool into the library tube in the Placed Samples area.
   a. Scan or type the barcode of the library tube into the Library Tube field.
   b. Select Record Details.
   On exit of the Placement screen, the Validate Library Tube Barcode automation checks that the library tube barcode conforms to the barcode mask [A-Za-z]{2}[0-9]{7}-[A-Za-z]{3}. If not, an error message displays.
4. On the Record Details screen, the Reagent Lot Tracking section tracks the NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. To add and activate reagent lots, complete the following steps:
   a. In a new browser tab, open an additional instance of Clarity LIMS.
      - Clarity LIMS v5.0: Navigate to the Consumables configuration screen.
      - Clarity LIMS v4.2: Navigate to the Reagents and Controls screen.
   b. Select the NaOH reagent kit and select New Lot.
   c. Enter the lot details and select Save.
   d. Set the Status of Reagent Lot toggle switch to Active.
   e. Repeat this process to add and activate Resuspension Buffer and Tris-HCl reagent lots.
   f. Close this browser tab and return to your original working tab. Refresh the page.

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5. In the Reagent Lot Tracking section, select from the active lots displayed in each drop-down list. The fields displayed in the Step Details section are used to generate the sample sheet and run recipe files. Some of these fields are autopopulated and some must be completed manually. See the following table for details.

Table 296 Fields Displayed on Record Details Screen of Denature and Dilute (NovaSeq 6000 v2.1) Step

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. No spaces.
BaseSpace Sequence Hub Configuration	Select from preset options: Not Used, Run Monitoring Only, or Run Monitoring and Storage.
Workflow	Automatically populated with preset— GenerateFASTQ.
Workflow Type	Select from preset options: No Index, Single Index, or Dual Index.
Index Read 1	Select from preset options: 0, 6, or 8—or type a value between 0 and 20.
Index Read 2	Select from preset options: 0, 6, or 8—or type a value between 0 and 20.
Paired End	Select from preset options: True or False.
Read 1 Cycles	Select from preset options: 251, 151, 101, or 51—or type a value between 1 and 251.
Read 2 Cycles	Select from preset options: 251, 151, 101, or 51—or type a value between 1 and 251.
Use Custom Read 1 Primer	Select if applicable. LIMS v5.0.5 and later: See the Configuration Update documentation.
Use Custom Read 2 Primer	Select if applicable. LIMS v5.0.5 and later: See the Configuration Update documentation.
Use Custom Index Read 1 Primer	Select if applicable. LIMS v5.0.5 and later: See the Configuration Update documentation.
Reverse Complement Workflow	Select Yes (by default) for v1.5 reagents in NovaSeq Control Software v1.7 and onwards. Select No for v1 reagents.
UMI - Read 1 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 1. Leave blank if not applicable.

Field	Value
UMI - Read 2 Length	Enter the length of the Unique Molecular Identifiers (UMI) in Read 2. Leave blank if not applicable.
UMI - Read 1 Start From Cycle	Enter the cycle number that Read 1 starts from. Leave blank if not applicable.
UMI - Read 2 Start From Cycle	Enter the cycle number that Read 2 starts from. Leave blank if not applicable.
Sample Sheet Path	Automatically populated after the sample sheet has been generated.

6. On the Record Details screen, select Generate Sample Sheet and Run Recipe. This selection triggers the automation script: The sample sheet and JSON files are generated and attached to the placeholders in the Files area of the Record Details screen.
   The Sample Sheet Path field is populated with the path to the sample sheet file.
7. Select Next Steps. This selection triggers the Prepare Files for NovaSeq automation, which does the following:
   - Copies the sample sheet and run recipe files to the location specified during installation. The NovaSeq instrument software uses these files to set up the run.
   - Sets the value of the next step to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step successfully.
8. On the Assign Next Steps screen, the Next Step for samples is prepopulated with Remove from workflow.
   Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.
9. Select Finish Step.
   On exit from the step, the Routing Script automation is triggered and samples are routed to Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.1).
   In Lab View, the pool of samples is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.1) step.

At this point in the workflow, the user interaction ends. Proceed to Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.1).

> Do not add samples to the Ice Bucket or start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.1) step. The integration does this action automatically.

Protocol 3: NovaSeq Xp (NovaSeq 6000 v2.1)

In this protocol, samples are pooled and added to lanes on the NovaSeq flow cell. The flow cell type is determined by the option selected in the Define Run Format (NovaSeq 6000 v2.1) step.

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The protocol contains three steps:

1. Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.1)
2. Dilute, Denature and ExAmp (NovaSeq 6000 v2.1)
3. Load to Flowcell (NovaSeq 6000 v2.1)

Step 1: Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.1)

1. In Lab View, locate the NovaSeq Standard (NovaSeq 6000 v2.1) protocol. The samples are queued for the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.1) step.
2. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucket and select Begin Work.
3. On the Pooling screen, create a pool by dragging samples into the Pool Creator. Only one pool must be created.
   a. Type a name for the pool or accept the default name (Pool #1).
   b. Select Record Details.
4. On exit from the Pooling screen, the Validate Inputs Flowcell Type and Single Pool is triggered. The automation checks the following details:
   - All samples in the pool have been assigned the same Flowcell Type.
   - Only one pool has been created.
5. On the Record Details screen in the Step Details area, complete the two required fields and one optional field as needed:
   - Number of Flowcells to Sequence—The value entered in this field is used in volume calculations to make sure that volumes are sufficient for the number of times the pool is sequenced.
   - Minimum Per Sample Volume (µl)—The value in this field is used to calculate how much of each sample is included in the pool. The field is prepopulated with the configured default value (5 µl), but the value can be edited if necessary.
   - % PhiX (2.5 nM) Spike-In—The value in this field is used to calculate the volume of PhiX v3 control to be included in the pool for the given percentage of spike-in. The field is optional and can be edited if necessary.

     Assuming the default Minimum Per Sample Volume (µl) value of 5, for a given batch:
     - If the smallest Per Sample Volume (µl) value is less than 5, the LIMS automatically assigns a value of 5 to the sample's Adjusted Per Sample Volume (µl) field.
     - Clarity LIMS then adjusts the Adjusted Per Sample Volume (µl) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.
6. In the Sample Details table, select the pool icon to view details on the pool composition.

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7. Select Calculate Volumes to trigger the Calculate Volumes automation, which performs volume calculations based on the selected Flowcell Type:
   - Calculates Bulk Pool Volume (µl) value.
   - Calculates the Per Sample Volume (µl) value to be added to the pool.
   - Calculates PhiX Volume (µl) value if % PhiX (2.5 nM) Spike-In has value.
   - Calculates the Total Sample Volume (µl) value.
   - If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (µl) value.
   - Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
   - The automation also generates the Calculation File (*csv) and attaches it to the step. This file contains volume information about the samples and RSB buffer to add to the pool. Select the file to download it, then open it in Excel.
8. Select Next Steps to trigger the Set Next Step automation. This automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol—Dilute, Denature and ExAmp (NovaSeq 6000 v2.1).
   On the Assign Next Steps screen, the next step for samples is already set to the next step in the workflow—Dilute, Denature and ExAmp (NovaSeq 6000 v2.1).
9. Select Finish Step.

At the end of this step, the pool of samples automatically advances to the Dilute, Denature and ExAmp (NovaSeq 6000 v2.1) step.

Step 2: Dilute, Denature and ExAmp (NovaSeq 6000 v2.1)

1. In Lab View, locate the NovaSeq Xp (NovaSeq 6000 v2.1) protocol. The pool of samples is queued for the Dilute, Denature and ExAmp (NovaSeq 6000 v2.1) step. Add the pool to the Ice Bucket.
2. On the Ice Bucket screen, set the number of derivatives to create (they are placed into the flow cell lanes) and select Begin Work.
   The Validate Inputs Flowcell Type automation checks that there is only one container input to the step.
3. On entry to the Record Details screen, the Calculate Volumes automation is triggered.
   This automation sets the following values based on the selected Flowcell Type:
   - NaOH Volume (µl)
   - Tris-HCl Volume (µl)
   - DPX1 Volume (µl)
   - DPX2 Volume (µl)
   - DPX3 Volume (µl)
   - Mastermix per Lane (µl)

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- The automation also generates the Calculation File (*csv) and attaches it to the step. This file contains information about the DPX Mastermix volume and the volume of Mastermix, NaOH, and Tris-HCl to add per working pool.

4. On the Record Details screen, the Reagent Lot Tracking section tracks the DPX1, DPX2, DPX3, NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. To add and activate reagent lots, complete the following steps:
   a. In a new browser tab, open an additional instance of Clarity LIMS.
      - Clarity LIMS v5: Navigate to the Consumables configuration screen.
      - Clarity LIMS v4: Navigate to the Reagents and Controls screen.
   b. Select one of the reagent kits listed and select New Lot.
   c. Enter the lot details and select Save.
   d. Set the Status of Reagent Lot toggle switch to Active.
   e. Repeat this process to add and activate the remaining reagent lots.
   f. Close this browser tab and return to your original working tab. Refresh the page.
5. On the Record Details screen in the Reagent Lot Tracking section, select from the active lots displayed in each drop-down list.
   - In the Step Details area, the DPX1, DPX2, and DPX3 reagent volume values are already populated. These values are set by a script and are not editable while running the step.
6. In the Sample Details table, select the pool icon to view details on the working pool composition.
   - The Bp Aliquot, Mastermix per lane, NaOH, and Tris-HCl volume values for each working pool are already populated. These values are set by a script and are not editable while running the step.
   - The working pool number is appended to the bulk pool name. This feature allows you to identify which working pools are derived from the same bulk pool quickly.
7. Select Next Steps.
   On the Assign Next Steps screen, the next step is already set to Load to Flowcell (NovaSeq 6000 v2.1).
8. Select Finish Step.

Step 3: Run Load to Flowcell (NovaSeq 6000 v2.1)

1. On the Ice Bucket screen in the Container Options panel, select the appropriate flow cell type from the Destination Container drop-down list and select Begin Work.
2. On the Placement screen, drag the pools from the left of the screen over into the Placed Samples area on the right.
   a. Scan or type the barcode of the flow cell into the Flow Cell field.
   b. Select Record Details.

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On exit of the Placement screen, the Validate Flowcell Barcode automation validates the container barcode.

3. The fields displayed in the Step Details section are used to generate the sample sheet and run recipe files. Some of these fields are autopopulated and some must be completed manually. See the following table for details.

Table 297 Fields Displayed on Record Details Screen of Load to Flowcell (NovaSeq 6000 v2.1) Step

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. No spaces.
BaseSpace Sequence Hub Configuration	Select from preset options: Not Used, Run Monitoring Only, or Run Monitoring and Storage.
Workflow	Automatically populated with preset - GenerateFASTQ.
Workflow Type	Select from preset options: No Index, Single Index, and Dual Index.
Index Read 1	Select from preset options: 0, 6, or 8—or type a value between 0 and 20.
Index Read 2	Select from preset options: 0, 6, or 8—or type a value between 0 and 20.
Paired End	Select from preset options: True or False.
Read 1 Cycles	Select from preset options: 251, 151, 101, or 51—or type a value between 1 and 251.
Read 2 Cycles	Select from preset options: 251, 151, 101, or 51—or type a value between 1 and 251.
Use Custom Read 1 Primer	Select if applicable. LIMS v5.0.5 and later: See the Configuration Update documentation.
Use Custom Read 2 Primer	Select if applicable. LIMS v5.0.5 and later: See the Configuration Update documentation.
Use Custom Index Read 1 Primer	Select if applicable. LIMS v5.0.5 and later: See the Configuration Update documentation.
Sample Sheet Path	Automatically populated after the sample sheet has been generated.
Library Tube Barcode	Scan the library tube barcode.

4. Select Generate Sample Sheet and Run Recipe. This selection triggers the automation script:
   - The sample sheet and JSON files are generated and attached to the placeholders in the Files area of the Record Details screen.
   - The Sample Sheet Path field is populated with the path to the sample sheet file.

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- If applicable, the Index Read 2 and Read 2 Cycles fields are populated.

5. Select Next Steps. This selection triggers the Prepare Files for NovaSeq automation, which copies the sample sheet and run recipe files to the location specified during installation. The NovaSeq instrument software uses these files to set up the run.
6. On the Assign Next Steps screen, the Next Step for samples is prepopulated with Mark protocol as complete.
7. Select Finish Step.

At this point in the workflow, the user interaction ends. The flow cell is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.1) step.

Proceed to Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.1).

Do not add samples to the Ice Bucket or start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.1) step. The integration does this action automatically.

Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.1)

This protocol contains a single fully automated step - AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.1).

The integration starts the step automatically and data from the run is parsed back into Clarity LIMS. No user interaction is required. However, you can open and review the various stages of the step in Clarity LIMS.

How the Integration Works

1. When initiating a run on the NovaSeq instrument, the NovaSeq Control Software (NVCS) looks for the sample sheet and run recipe (*.json file) on the shared network drive. The NVCS copies the sample sheet to the run directory.
   The NVCS uses the run recipe to start the sequencing run.
2. When the run starts, the instrument software creates a new run folder on the shared network drive and copies the following files into that folder:
   - `RunParameters.xml`
   - `RunInfo.xml`
   - LIMS/ <ContainerID>.json file (run recipe)
   - The Real-Time Analysis v3 (RTA3) software creates the InterOp folder.
3. The sequencing service detects the presence of the `RunParameters`, `RunInfo`, and JSON files and starts the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.1) step in Clarity LIMS.
4. As the run progresses, the InterOp data files are filled in.
5. At the end of the run, the NovaSeq creates the `CopyComplete.txt` file in the run folder, which indicates to the sequencing service that the run has completed and the data files are ready.

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6. The NovaSeq copies the run data files to the InterOp folder and the sequencing service triggers the Read InterOp Metrics automation. This automation reads the files, records the parsed metrics into Clarity LIMS, and completes the step in Clarity LIMS.

Parse Run Parameters and Generate Link to Run Folder Automation

The Parse Run Parameters and Generate Link to Run Folder automation is automatically triggered on entry to the Record Details screen. This automation parses the `RunParameters.xml` file attached on the Step Setup screen and populates the step UDFs/custom fields with extracted values. For details on this automation, including how the parsed fields map to the step UDFs/custom fields, see the *NovaSeq 6000 Integration User and Configuration Guide*.

Read InterOp Metrics Automation

Automatically triggered when the sequencing service detects the `CopyComplete.txt` file has been created, this automation parses the InterOp data generated by the run.

The `read_interop_metrics` script records summary metrics into the following global UDFs/custom fields in Clarity LIMS. The script fails if any field is not configured or cannot be written to.

- Yield PF (Gb) R1
- Yield PF (Gb) R2
- Reads PF (M) R1
- Reads PF (M) R2
- Cluster Density (K/mm^2) R1
- Cluster Density (K/mm^2) R2
- %PF R1
- %PF R2
- % Bases >=Q30 R1
- % Bases >=Q30 R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- % Aligned R1
- % Aligned R
- % Error Rate R1
- % Error Rate R2

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For details, see the user and configuration guide.

Set Next Steps Automation

This automation sets the next step for all samples to Mark protocol as complete. For details, see the user and configuration guide.

Troubleshooting

If an automation trigger does not appear to run its corresponding scripts, see the following articles:

- *Troubleshooting Automated Informatics* in the Clarity LIMS documentation, specifically the Automated Informatics section
- *Troubleshooting EPP / Automation* in the API > Clarity LIMS API Overview > API Advanced Topics section

If an error occurs that does not provide direction on how to proceed, complete the following steps:

1. Confirm the version of the installed Illumina NovaSeq Integration Package by running the following command:

```
rpm -qa | grep -i novaseq
```

   - For an on-premise installation, run the command on Clarity LIMS server command line. This command retrieves the version of both RPMs installed.
   - For a Software as a Service (SaaS) installation, run the command on the local server installation. This command retrieves the version of the remote extensions RPM installed.

2. If the error is related to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.1) step, there are two places to check for log file information:
   - If the step does not start, check the NovaSeqIntegrator.log log file written to:

```
/opt/gls/clarity/extensions/novaseq/SequencingService/NovaSeqIntegrator.log
```

     - This log file is written to this path wherever the remote extensions RPM was installed.
   - If the step starts but does not complete, open the step in Clarity LIMS (it can be found via a search). On the Record Details screen, the log file is attached to a placeholder called Log File. If you are unable to reach the Record Details screen, or if the file does not contain an error, review the `NovaSeqIntegrator.log` described previously.
3. Contact the Clarity LIMS support team, supplying the relevant information from the troubleshooting steps already performed.

NovaSeq 6000 Integration v2.3 Release Notes

The NovaSeq 6000 Integration v2.3 has the following compatibility:

- Clarity LIMS v4.2 or later, until v6.0. Not compatible with v6.0 or later.
- NGS Extensions Package v5.20 or later.
- NovaSeq Control Software (NVCS) v1.7.0.

- Illumina Bcl2FastQ v2.20.

Features to Note for this Release

This release of Clarity LIMS integration package provides:

- Support for the NovaSeq Control Software v1.7.0 with v1.5 reagents.
- Support for asymmetric sequencing read length.
- Support for reverse complement workflow in sample sheet generation.
- Support for Unique Molecular Identifiers (UMI) configuration that is populated in sample sheet.
- Updated preconfigured workflow (NovaSeq 6000 v2.1).
- Support SPrime (SP) flow cell in the preconfigured workflow.
- PhiX spike-in volume calculation script.
- Update to the latest flow cell barcode mask.
- New secret management mechanism.

Defects Fixed

- Fixed the 'NULL' value for custom primer settings in JSON recipe issue.
- Fixed the lane number discrepancy issue in Automated - NovaSeq Run step.

Known Issues

Minor bug in SecretUtil v1.0.0 that causes some NGS automation scripts to fail if the command does not have -l parameter. This will be fixed in the SecretUtil v1.0.1 release (Dec 2020). Until then, add "-l" in the automation script to bypass the bug i.e. bash -l -c "……". THe automation scripts affected are:

- assignQC
- assignQCForDemuxStep
- parseBioAnalyzer
- parseCSV
- place_samples_by_robot_file
- parseXmlBySampleNam

Related Documentation

The following documents are available on the Clarity LIMS NovaSeq Integrations download page.

- *NovaSeq 6000 Integration v2.3 User and Configuration Guide*
- *NovaSeq 6000 Integration v2.3 Validation and Troubleshooting Guide*

Configuration Update for Flowcell Barcode Suffix Change

The barcode suffix change is a standard manufacturing process that occurs when the barcodes with the current suffix have run out. In the case that all 9 digit barcodes for a particular product have been consumed by standard production, digit number 9 will increment with the following pattern:

X, Y, 2, 3, 5, 7

For example, the suffix for the S4 flowcell will increment from DSXX → DSXY → DSX2, and so on.

The same barcode update process will occur in the future for all flowcell types (i.e., SP, S1, S2, and S4).

For existing NovaSeq integration users, the 'Validate Flowcell Barcode' automation script must be updated to reflect this potential future change to avoid integration issues. Please follow the instructions below to update.

To resolve this issue, update the Validate Flowcell Barcode automation/EPP script associated with the following master step/process type:

- Load to Flowcell (NovaSeq 6000 v2.0)

Clarity LIMS v5.x Configuration

1. Sign in to Clarity LIMS with System Administrator permissions.
2. In the Configuration area, select the Automation tab, and then select the Validate Flowcell Barcode script.
3. Delete the content of the existing scripts and replace with the updated scripts provided below.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \ -exp 'if (input.::Flowcell Type:: == ::SP::
&& !output.container.name.matches( ::[A-Za-z0-9]{5}DRX[XY2357]:: ) ) {fail (
::Invalid Flowcell Barcode. Please verify and try again.:: ) } ; if
(input.::Flowcell Type:: == ::S1:: && !output.container.name.matches( ::[A-Za-
z0-9]{5}DRX[XY2357]:: ) ) {fail ( ::Invalid Flowcell Barcode. Please verify and
try again.:: ) } ; if (input.::Flowcell Type:: == ::S2:: &&
!output.container.name.matches( ::[A-Za-z0-9]{5}DMX[XY2357]:: ) ) {fail (
::Invalid Flowcell Barcode. Please verify and try again.:: ) } ; if
(input.::Flowcell Type:: == ::S4:: && !output.container.name.matches( ::[A-Za-
z0-9]{5}DSX[XY2357]:: ) ) {fail ( ::Invalid Flowcell Barcode. Please verify and
try again.:: ) } ; output.::Flowcell Type:: = input.::Flowcell Type:: ;
output.::Loading Workflow Type:: = input.::Loading Workflow Type::' \ -log
{compoundOutputFileLuid2}"
```

4. Select Save.

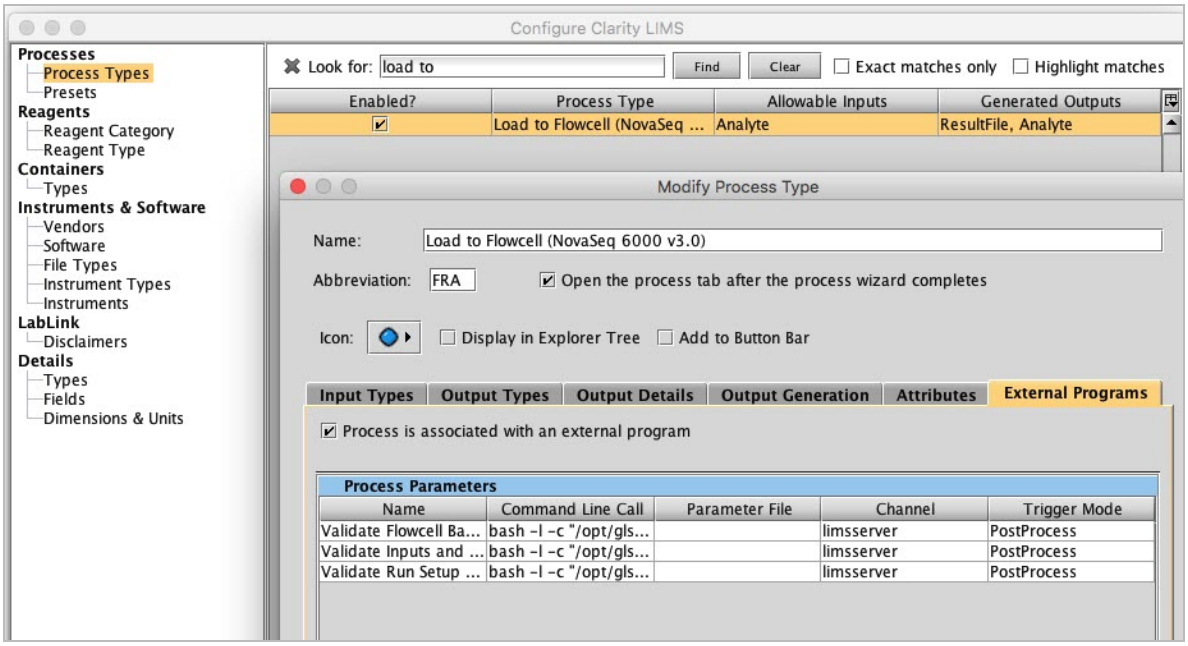
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5. If using a customized Validate Flowcell Barcode script, only replace the regex portion of individual flowcell type in the script.

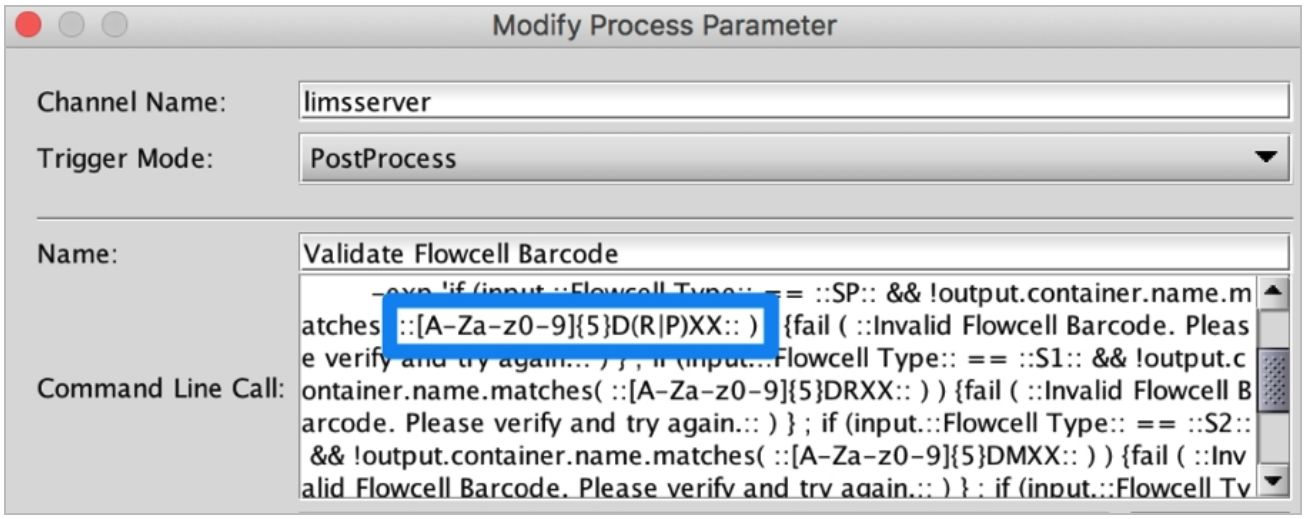
Flowcell Type	Existing regex	Updated regex
SP	[A-Za-z0-9]{5}DRXX	[A-Za-z0-9]{5}DRX[XY2357]
S1	[A-Za-z0-9]{5}DRXX	[A-Za-z0-9]{5}DRX[XY2357]
S2	[A-Za-z0-9]{5}DMXX	[A-Za-z0-9]{5}DMX[XY2357]
S4	[A-Za-z0-9]{5}DSXX	[A-Za-z0-9]{5}DSX[XY2357]

Clarity LIMS v4.2 Configuration

1. Log into the Operations Interface.
2. Select Configuration and then select Process Types.
   a. Search for Load to Flowcell process type and select the External Programs tab.
   b. Edit the Validate Flowcell Barcode process parameter.



   c. Replace the regex portion of the individual flowcell type in the script, as shown in the table for LIMS v5.x, above.



d. After all replacements are made, select Save in both the Modify Process Parameter and Modify Process Type windows.

e. Select Done.

Configuration Update for NovaSeq Integration with Clarity LIMS v5.0.5 and Later

In Clarity LIMS v5.0.5 and later, a change in custom field configuration causes a minor issue in the IlluminaNovaSeq Integration (all versions).

The following instructions resolve this issue through simple configuration changes. Contact the IlluminaSupport team if you need help with this configuration update.

Configuration Update Summary

Update the configuration for two master steps:

- Dilute and Denature (NovaSeq 6000 v2.0)
- Load to Flowcell (NovaSeq 6000 v2.0)

On each master step, change the default setting for the following three master step fields:

- Use Custom Read 1 Primer
- Use Custom Read 2 Primer
- Use Custom Index Read 1 Primer

Configuration Update Steps

1. Log in to Clarity LIMS with System Administrator permissions.

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2. In the Configuration area, click Custom Fields, and then click on the Master Step Fields tab.
3. Select the Dilute and Denature (NovaSeq 6000 v2.0)master step and click the arrow to expand the list of configured custom fields.
4. Select the Use Custom Read 1 Primercustom field.
5. In the Field Details area on the right, under Additional Options, notice that the Default is set to None Set.
6. Change the Default value to No.
7. Click Save.
8. Repeat steps 4 to 6 for the Use Custom Read 2 Primer and Use Custom Index Read 1 Primer fields, saving your changes for each field.
9. In the master fields list, select the Load to Flowcell (NovaSeq 6000 v2.0)and repeat steps 3 to 7 to configure the same three fields.

Illumina NovaSeq 6000 Integration v2.2.2 Release Notes

Compatibility:

- BaseSpace Clarity LIMS v4.2 or later, though not compatible with v6.0 and later
- NGS Extensions Package v5.15 or later
- BaseSpaceLIMS-AutomationWorker 0.1.0 (LIMS v4.2) or v1.0 (LIMS v5.0)

Illumina NovaSeq Software Compatibility: NovaSeq Control Software (NVCS) v1.5.0

Features to note for this release

This release of BaseSpace Clarity LIMS integration with the Illumina NovaSeq 6000 system fixes an issue related to network mount 2folder write permission.

Known issues

NovaSeq Control Software is unable to parse 'NULL' value from JSON recipe file generated by BaseSpace Clarity LIMS.

If you are upgrading from NovaSeq integration v2.1 or v2.2, you will need to update the configuration. Instructions are provided in the Configuration Update Guide.

Documentation

For detailed information related to this release, see the latest documentation in the Illumina NovaSeq Integration v2.0 section.

NovaSeq 6000 Integration v2.2.1 Configuration and User Interaction

The Illumina NovaSeq 6000 Integration Package v2.2.1 supports the integration of BaseSpace Clarity LIMS to Illumina NovaSeq 6000 sequencers.

This *User and Configuration Guide* describes the integration and includes information about the preconfigured protocols, steps, and automations; installed components; and configuration requirements, rules, and constraints.

For instructions on validating and troubleshooting the Illumina NovaSeq 6000 Integration, see the *NovaSeq 6000 Integration v2.2.1 - Validation and Troubleshooting Guide*.

Note:

The configuration provided in this integration has been established to support NovaSeq 6000 lab processes. Any configuration changes to protocols or workflows - including renaming protocols, steps, and fields - could break process.

Overview

The integration includes the following functionality:

- Preconfigured NovaSeq 6000 v2.0 workflow that maps to lab protocols and instrument runs.
- Preconfigured NovaSeq Standard (NovaSeq 6000 v2.0) protocol that supports the loading of pooled libraries into a library tube.
- Preconfigured NovaSeq Xp (NovaSeq 6000 v2.0) protocol that supports individual lane loading on the NovaSeq.
- Automated generation of a sample sheet for use with bcl2fastq2 v2.19 analysis software.
- Automatic generation of run recipe file (JSON format) that is automatically uploaded to the sequencing instrument and used to set up and initiate the run.
- Automated tracking of the NovaSeq sequencing run and parsing of run statistics (per run per lane) into the LIMS, including:Progress and metrics of sequencing run
  - Per-instrument sequencing runs (tracked as part of the Run ID field value)
  - Sequencing run parameters
  - Real Time Analysis v3 (RTA3) run directory location and other run specific information
- Preconfigured NovaSeq Validation Library Prep (NovaSeq 6000 v2.0) workflow used for validation purposes only. The workflow contains a single-step protocol that models the library prep required to produce normalized libraries that are ready for the NovaSeq 6000 v2.0 workflow. For details, see the *NovaSeq 6000 Integration v2.2.1 - Validation and Troubleshooting Guide*.

Information on these features is provided in the following sections.

Prerequisites and assumptions

It is assumed that samples enter the NovaSeq 6000 v2.0 workflow as normalized libaries. That is, before they are assigned to the workflow:

- Samples have been accessioned into the LIMS.
- Samples have been run through QC and library prep.
- Samples have been normalized, and the value is captured in a field called Normalized Molarity (nM).

For details on sample accessioning, see the following articles in the LIMS Documentation (Samples section):

- *Sample Accessioning*
- *Using Sample Lists to Upload and Modify Samples*

Samples can be assigned to the NovaSeq 6000 v2.0 workflow automatically (using a routing script), or manually from the Projects and Samples dashboard (see the *Assigning Samples to Workflows* article).

Workflows, protocols, and steps

The Illumina NovaSeq 6000 Integration Package v2.2.1 includes two workflows:

- NovaSeq 6000 v2.0
- NovaSeq Validation Library Prep (NovaSeq 6000 v2.0)

Table 1 lists and describes the protocols and steps included in these workflows.

Table 1: Illumina NovaSeq 6000 Integration Package v2.2.1 protocols and steps

NovaSeq Validation Library Prep (NovaSeq 6000 v2.0) workflow

Protocol name	Protocol purpose	Steps included in protocol
NovaSeq Validation Library Prep (NovaSeq 6000 v2.0)	Included for validation purposes only, this protocol models the library prep steps required to advance samples to the Run Format (NovaSeq 6000 v2.0) protocol. The protocol contains a single step - NovaSeq Validation Library Prep (NovaSeq 6000 v2.0). At the end of this step, a routing script sends the samples to the first step of the NovaSeq 6000 v2.0 workflow - Define Run Format (NovaSeq 6000 v2.0).	1. NovaSeq Validation Library Prep (NovaSeq 6000 v2.0)

NovaSeq 6000 v2.0 workflow

Protocol name	Protocol purpose	Steps included in protocol

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NovaSeq Validation Library Prep (NovaSeq 6000 v2.0) workflow

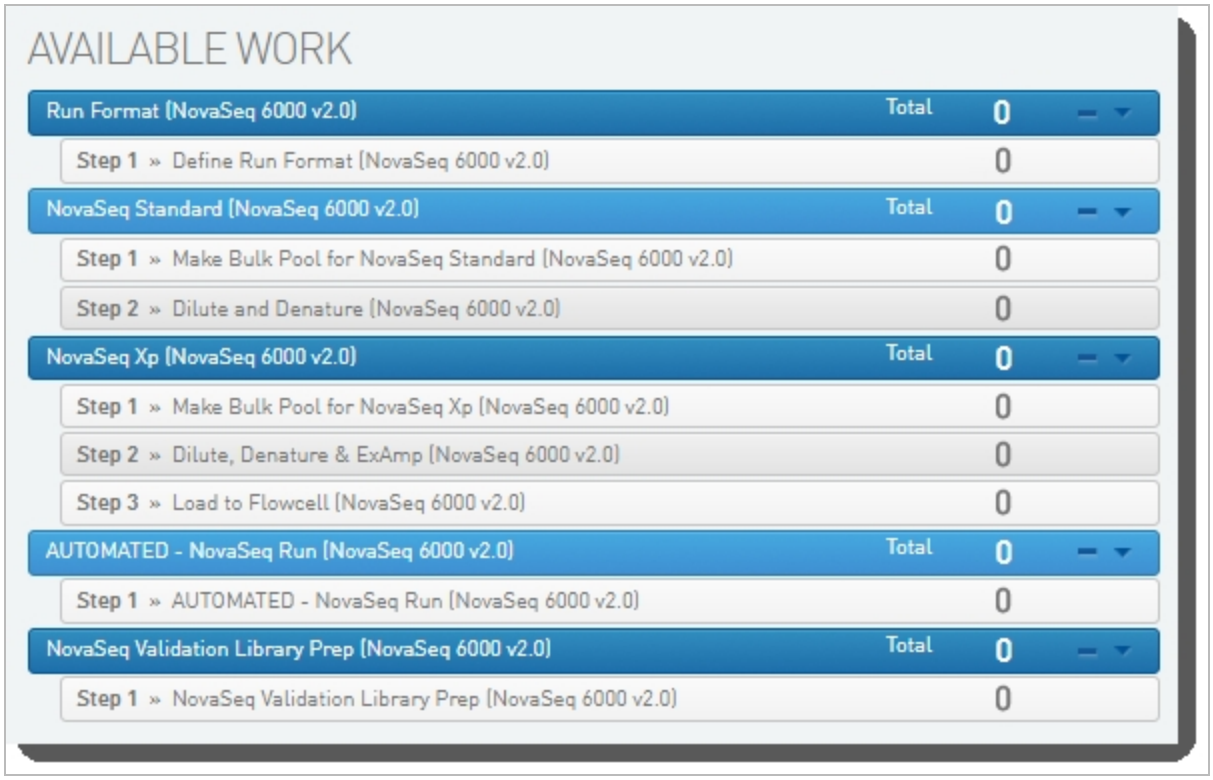
Run Format (NovaSeq 6000 v2.0)	Allows for the assignment of per sample values for Loading Workflow Type, Normalized Molarity, Flowcell Type, and Final Loading Concentration (pM): • Loading Workflow Type: User selects from NovaSeq Standard or NovaSeq Xp. • Normalized Molarity: User enters a value for each sample. • Flowcell Type: User selects from options S1, S2, and S4. • Final Loading Concentration (pM): User selects from the options 225 (PCR-free workflows) or400 (Nano workflows), or may enter a different value. Compares each sample's Normalized Molarity value with the Minimum Molarity value. Routing script sends samples to the NovaSeq Standard or NovaSeq Xp protocol, according to the selected Loading Workflow Type. Samples with Normalized Molarity less than Minimum Molarity are removed from the workflow.	1. Define Run Format (NovaSeq 6000 v2.0
NovaSeq Standard (NovaSeq 6000 v2.0	Samples are pooled and added to the library tube in preparation for the NovaSeq run. Samplesheet and run recipe files are generated. Routing script sends library tube to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) protocol.	1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0) 2. Dilute and Denature (NovaSeq 6000 v2.0)

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NovaSeq Validation Library Prep (NovaSeq 6000 v2.0) workflow

NovaSeq Xp (NovaSeq 6000 v2.0)	Samples are pooled and added to lanes on the NovaSeq flow cell. The flow cell type is determined by the option selected in the Define Run Format (NovaSeq 6000 v2.0) step. Samplesheet and run recipe files are generated. Samples are queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) protocol.	1. Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.0) 2. Dilute, Denature and ExAmp (NovaSeq 6000 v2.0) 3. Load to Flowcell (NovaSeq 6000 v2.0)
AUTOMATED - NovaSeq Run (NovaSeq6000 v2.0)	All samples complete the workflow by going through this protocol. This protocol contains 1 fully-automated step. The user must not add samples to the Ice Bucket or start the step. The integration will do this automatically.	1. AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0)

The following image show the protocols and their steps displayed in the LIMS Lab View.



Validation workflow

The NovaSeq Validation Library Prep (NovaSeq 6000 v2.0) workflow allows for validation of the system after installation is complete. For details, see the *NovaSeq 6000 Integration v2.2.1 - Validation and Troubleshooting Guide*.

Step automations

Table 2 lists the step automations included in the NovaSeq 6000 v2.0 workflow. Automation details are provided in the sections following the table.

For details on the validation workflow, see the *NovaSeq 6000 Integration v2.2.1 - Validation and Troubleshooting Guide*.

Table 2: NovaSeq 6000 v2.0 workflow step automations

Protocol	Step	Automation
1. Run Format (NovaSeq 6000 v2.0)	1. Define Run Format (NovaSeq 6000 v2.0)	• Routing Script • Set Next Step
2. NovaSeq Standard (NovaSeq 6000 v2.0)	1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0)	• Calculate Volumes • Set Next Step • Validate Inputs Flowcell Type and Single Pool • Validate Unique Indexes *Not used. This functionality is handled by the core LIMS configuration for pooling.*
	2. Dilute and Denature (NovaSeq 6000 v2.0)	• Generate Sample Sheet and Run Recipe • Prepare Files for NovaSeq • Routing Script • Validate Library Tube Barcode • Validate Single Input
3. NovaSeq Xp (NovaSeq 6000 v2.0)	1. Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.0)	• Calculate Volumes • Set Next Step • Validate Inputs Flowcell Type and Single Pool • Validate Unique Indexes *Not used. This functionality is handled by the core LIMS configuration for pooling.*
	2. Dilute, Denature and ExAmp (NovaSeq 6000 v2.0)	• Calculate Volumes • Set Next Step • Validate Inputs Flowcell Type
	3. Load to Flowcell (NovaSeq 6000 v2.0)	• Generate Sample Sheet and Run Recipe • Prepare Files for NovaSeq • Validate Flowcell Barcode • Validate Inputs and Selected Container

Protocol	Step	Automation
4. AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0)	1. AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0)	• Parse Run Parameters and Generate Link to Run Folder • Read InterOp Metrics • Set Next Steps

The sections below describe the configuration installed with the Illumina NovaSeq 6000 Integration.

Note:
The Illumina NovaSeq 6000 Integration Package v2.2.1 supports BaseSpace Clarity LIMS v4.2 and v5.0.
As of LIMS v5, the term user-defined field (UDF) has been replaced with custom field in the user interface (the API resource is still called udf). There are two types of custom fields:

- Master step fields - configured on master steps. These fields only apply to the master step on which they are configured, and the steps derived from those master steps.
- Global fields - configured on entities such as submitted sample, derived sample, measurement, etc. These fields apply to the whole LIMS system.

Protocol 1: Run Format (NovaSeq 6000 v2.0)

This protocol sets the Loading Workflow Type and allows the user to choose the appropriate Flowcell Type and Final Loading Concentration(pM). At the end of the protocol, a routing script sends the normalized libraries to either the NovaSeq Standard (NovaSeq 6000 v2.0) or the NovaSeq Xp (NovaSeq 6000 v2.0) protocol.

This protocol contains one step: Define Run Format (NovaSeq 6000 v2.0), described below.

Step 1: Define Run Format (NovaSeq 6000 v2.0)

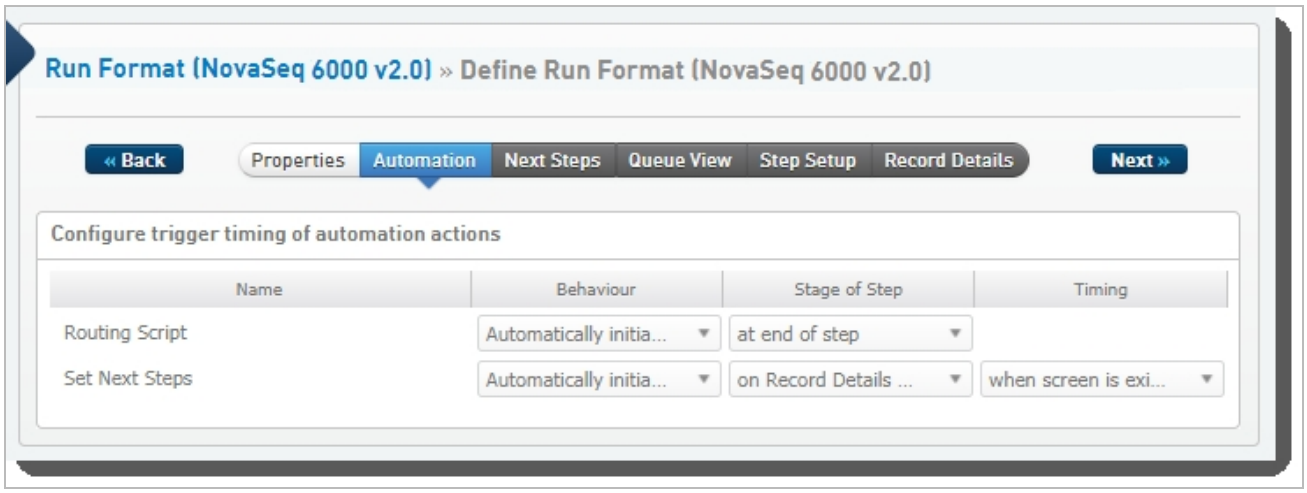
Step input: NTP (normalized libraries)

Step output: None

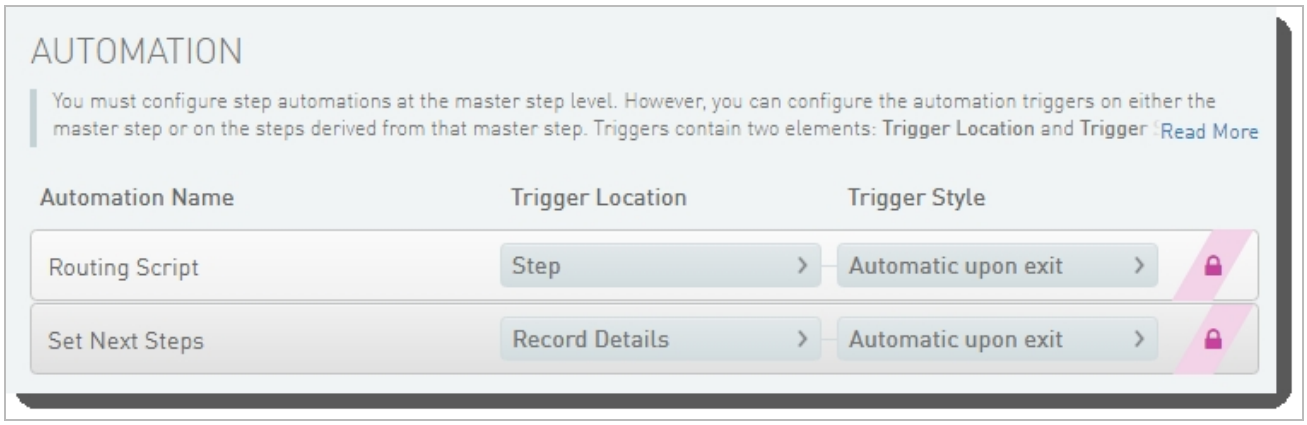
The following automations are configured on this step:

- Routing Script
- Set Next Steps

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Define Run Format (NovaSeq 6000 v2.0) automation configuration - LIMS v4.2



Define Run Format (NovaSeq 6000 v2.0) automation configuration - LIMS v5

These automations are described below, in the order in which they are triggered at run time

Set Next Steps

Automatically triggered on exit of the Record Details screen, this automation does the following:

- Sets the next step for samples to REMOVE:

```
nextStep = ::REMOVE::
```

- Calculates the Minimum Molarity using the following formula:

```
input.::Minimum Molarity (nM):: = (5 * input.::Final Loading Concentration
(pM)::)/1000
```

- Checks Normalized Molarity value. For samples with no Normalized Molarity value (i.e., empty value, not including 0), generates an error message informing the user that the field cannot be empty:

```
if (!input.hasValue(::Normalized Molarity (nM)::)) { fail(::The Normalized
Molarity cannot be empty.::) ; }
```

- Compares each sample's Normalized Molarity value with the Minimum Molarity value.

- If Normalized Molarity value is lower than the Minimum Molarity value, sets the sample's Loading Workflow Type to [Remove from workflow] and records a message in the Warning field for the sample:

```
else if (input.::Normalized Molarity (nM):: < input.::Minimum Molarity (nM)::) {
input.::Warning:: = ::The Normalized Molarity is too low.:: ; input.::Loading
Workflow Type:: = ::[Remove from workflow]:: ; } else { input.::Warning:: =
::n/a:: }
```

  At this point the user has two options:

  – Correct the Normalized Molarity value on the Record Details screen. User will also need to edit the Loading Workflow Type field and set it to NovaSeq Standard or NovaSeq Xp, as applicable.
  – Complete the protocol without correcting the Normalized Molarity value. In this case, those samples will be removed from the workflow.

Routing Script

Automatically triggered on exit of the step, this automation invokes the changeWorkflow script, which routes step inputs appropriately.

- Samples with Loading Workflow Type field value = NovaSeq Standard are routed to the NovaSeq 6000 v2.0 workflow and queued for the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0) step.
- Samples with Loading Workflow Type field value = NovaSeq Xp are routed to the NovaSeq 6000 v2.0 workflow and queued for the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.0) step.

Default automation command line:

```
bash -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -u {username} -p {password} -i {stepURI:v2} -l
{compoundOutputFileLuid0} script:changeWorkflow \\--FIELD_NAME 'Loading Workflow
Type' \--FIELD_VALUE 'NovaSeq Standard' \--WORKFLOW 'NovaSeq 6000 v2.0' \--STEP
'Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0)' \--INPUTS_OR_OUTPUTS
'INPUTS' \\--FIELD_NAME 'Loading Workflow Type' \--FIELD_VALUE 'NovaSeq Xp' \--
WORKFLOW 'NovaSeq 6000 v2.0' \--STEP 'Make Bulk Pool for NovaSeq Xp (NovaSeq 6000
v2.0)' \--INPUTS_OR_OUTPUTS 'INPUT
```

Step UDFs/custom fields

There are two fields defined on the Define Run Format (NovaSeq 6000 v2.0) step.

These fields are shown below in the BaseSpace Clarity LIMS v5 Configuration area (Custom Fields > Master Step Fields tab). See Table 3 for LIMS v4.2 and LIMS v5 field configuration details.



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Table 3: Define Run Format (NovaSeq 6000 v2.0) step UDF / custom field configuration

Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
Comment	Multi-line Text	• Required: Yes • Read Only: No	None
Instruction	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: Yes	• Default: Add Flowcell Type and Loading Workflow Type below

Global UDFs/custom fields

Table 4 lists the global UDFs / custom fields that are configured to display on the Define Run Format (NovaSeq 6000 v2.0) step.

Table 4: Global UDF / custom field configuration

Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
Configured on Analyte in LIMS v4.2 and Derived Samplein LIMS v5:			
Adjusted Per Sample Volume (ul)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Final Loading Concentration (pM)	Numeric (v4.2) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 225 • 400 • Decimal Places Displayed: 0
Flowcell Type	Single-line Text (v4.2) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• S1 • S2 • S4
Loading Workflow Type	Single-line Text (v4.2) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
Minimum Molarity (nM)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2
Normalized Molarity (nM)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2

Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
Per Sample Volume (ul)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Warning	Single-line Text (v4.2) Text Dropdown (v5)	• Required: No • Read Only: Yes • Custom Entries: Yes	• The Normalized Molarity (nM) is too low. • n/a

User interaction

1. On the Record Details screen, the Sample Details table contains the following fields that must be completed (values can vary across samples):Loading Workflow Type: User selects NovaSeq Standard or NovaSeq Xp from the drop-down list.
    - Flowcell Type: User selects S1, S2, or S4 from the drop-down list.
    - Final Loading Concentration (pM): User selects from the two preset options - 225 (for PCR-free workflows) or400 (for Nano workflows), or may enter a different value.
    - Normalized Molarity: User enters a value for each sample.
    - User clicks Next Steps. This triggers the Next Steps automation, which does the following: Sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value, and requires it in order to successfully advance samples to the next step.
    - Calculates the Minimum Molarity.
    - Checks Normalized Molarity value. For samples with no Normalized Molarity value (i.e., empty value, not including 0), generates an error message informing the user that the field cannot be empty.
    - Compares each sample's Normalized Molarity value with the Minimum Molarity value.
2. On the Assign Next Steps screen:
    - In the Sample Details table, the Next Step for all samples is pre-populated with Remove from workflow - regardless of the Loading Workflow Type  *The user must not change this value! If Next Step is not set to Remove from workflow, the routing script will not be able to route samples correctly.*
    - For samples whose Normalized Molarity value was found to be lower than the Minimum Molarity value, the Loading Workflow Type is set to Remove from workflow and a message is recorded in the Warning field for the sample. At this point the user has two options:

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- Return to the Record Details screen and adjust the Normalized Molarity value so that it equals or exceeds the Minimum Molarity value. User will also need to set the Loading Workflow Type to NovaSeq Standard.
- Complete the protocol without correcting the Normalized Molarity value. In this case, the samples in question will be removed from the LIMS workflow.

3. User clicks Finish Step. The Routing Script automation is triggered:
    - Samples whose Loading Workflow Type is set to Remove from workflow (i.e., whereNormalized Molarity value is lower than the Minimum Molarity) are removed from the LIMS workflow.
    - Samples whose Loading Workflow Type is set to NovaSeq Standard are routed to the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0) step. This is the first of two steps in the NovaSeq Standard (NovaSeq 6000 v2.0) protocol.
    - Samples whose Loading Workflow Type is set to NovaSeq Xp are routed to the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.0) step. This is the first of three steps in the NovaSeq Xp (NovaSeq 6000 v2.0) protocol.

Protocol 2: NovaSeq Standard (NovaSeq 6000 v2.0)

Samples are routed to this protocol if their Loading Workflow Type value is set to NovaSeq Standard. Samples are pooled and added to a library tube in preparation for the NovaSeq run.

At the end of this protocol, a routing script sends the library tube to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) protocol.

This protocol contains two steps, described below:

- Step 1: Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0)
- Step 2: Dilute and Denature (NovaSeq 6000 v2.0)

Step 1: Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0)

In this step, user manually places libraries into a single pool. Resuspension buffer and reagents are added.

Note:

User must only create only one pool per step.

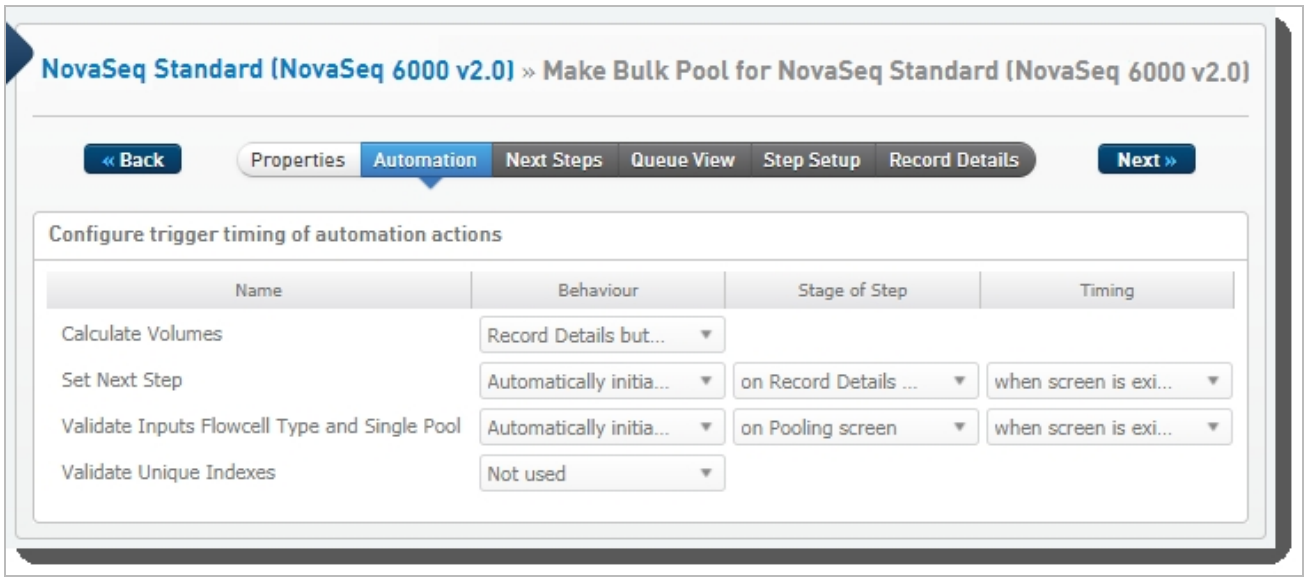
Step input: NTP (normalized libraries)

Step output: Bulk pool

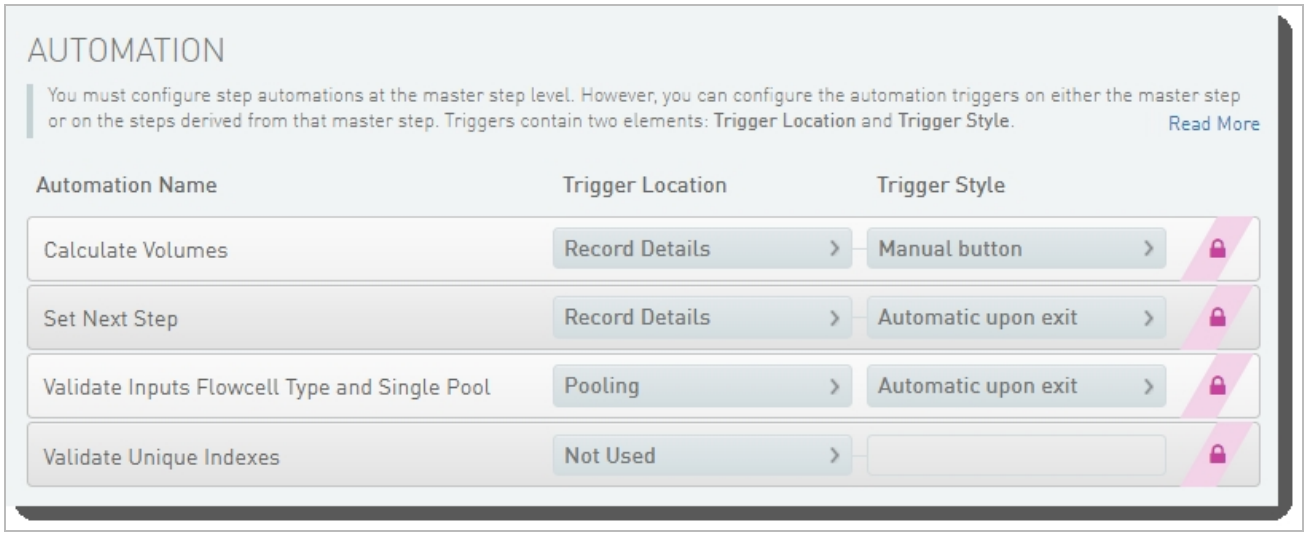
The following automations are configured on the step:

- Calculate Volumes
- Set Next Step
- Validate Inputs Flowcell Type and Single Pool

- Validate Unique Indexes*(Not used. This functionality is handled by the core LIMS configuration for pooling.)*



Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0) automation configuration - LIMS v4.2



Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0) automation configuration - LIMS v5

These automations are described below, in the order in which they are triggered at run time.

Validate Inputs Flowcell Type and Single Pool

Automatically triggered on exit of the Pooling screen, this automation checks that:

- All samples in the pool have the same Flowcell Type assigned to them.
- Only one pool has been created.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/novaseq/novaseq-extensions.jar script:validate_flowcell_for_input_pools -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -validateSingleOutput true -poolType bulk
```

Calculate Volumes

Automatically triggered when the user clicks the Calculate Volumes button on the Record Details screen, this automation:

- Calculates the number of samples in the pool:

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: + 1
```

- Sets the value of the Bulk Pool Volume (ul) field, based on the selected Flowcell Type:

```
if (input.::Flowcell Type:: == ::S1::) { step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 100 + 30 }; if (input.::Flowcell Type:: == ::S2::) { step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 150 + 30 }; if (input.::Flowcell Type:: == ::S4::) { step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 310 + 30 };
```

  Calculates the Per Sample Volume (ul) to be added to the pool:

```
input.::Per Sample Volume (ul):: = ( ( (input.::Final Loading Concentration (pM):: * 5 / 1000) / input.::Normalized Molarity (nM):: ) * step.::Bulk Pool Volume (ul):: ) / step.::Number of Samples in Pool::
```

  Note:
  To ensure accurate pipetting of each sample in a pool for sequencing, the Per Sample Volume (ul) value must be equal to or higher than the Minimum Per Sample Volume (ul) (default value set at 5, may be edited by the user).
  Assuming the default Minimum Per Sample Volume (ul) value of 5, for a given batch:
  1. If the smallest Per Sample Volume (ul) value is less than 5, the LIMS automatically assigns a value of 5 to the sample's Adjusted Per Sample Volume (ul) field.
  2. The LIMS then adjusts the Adjusted Per Sample Volume (ul) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

- Calculates the Total Sample Volume (ul) field value:

```
step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: + input.::Adjusted Per Sample Volume (ul)::
```

- If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (ul) field value:

```
if (step.::Total Sample Volume (ul):: >= step.::Bulk Pool Volume (ul)::) {output.::RSB Volume (ul):: = 0} else {output.::RSB Volume (ul):: = step.::Bulk Pool Volume (ul):: - step.::Total Sample Volume (ul)::} ;
```

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs:

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```
output.::Flowcell Type::= input.::Flowcell Type::;output.::Loading Workflow
Type::= input.::Loading Workflow Type::;
```

- Sets the Volume of Pool to Denature (ul) value and calculates NaOH Volume (ul) and Tris-HCl Volume (ul) values, based on the Flowcell Type:

```
if ( input.::Flowcell Type:: == ::S1:: ) { output.::Volume of Pool to Denature
(ul):: = 100 ; output.::NaOH Volume (ul):: = 25.00 ; output.::Tris-HCl Volume
(ul):: = 25.00 } ; if ( input.::Flowcell Type:: == ::S2:: ) {output.::Volume of
Pool to Denature (ul):: = 150 ; output.::NaOH Volume (ul):: = 37.50 ;
output.::Tris-HCl Volume (ul):: = 37.50 } ; if ( input.::Flowcell Type:: ==
::S4:: ) { output.::Volume of Pool to Denature (ul):: = 310 ; output.::NaOH
Volume (ul):: = 77.50 ; output.::Tris-HCl Volume (ul):: = 77.50 }
```

- Uses the NovaSeq_Standard_Bulk_Pool1.csv, NovaSeq_Standard_Bulk_Pool2.csv and NovaSeq_Standard_Bulk_Pool3.csv template files to generate a single CSV file containing information about the pool and the samples it contains. The generated file is available for download by the user on the Step Setup screen of the following step - Dilute and Denature (NovaSeq 6000 v2.0).

```
script:driver_file_generator \ -t
/opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Standard_Bulk_Pool1.csv \ -
o 1.csv \ script:driver_file_generator \ -t
/opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Standard_Bulk_Pool2.csv \ -
o 2.csv \ script:driver_file_generator \ -t
/opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Standard_Bulk_Pool3.csv \ -
o 3.csv \&& cat 1.csv 2.csv 3.csv > {compoundOutputFileLuid0}.csv
```

Set Next Step

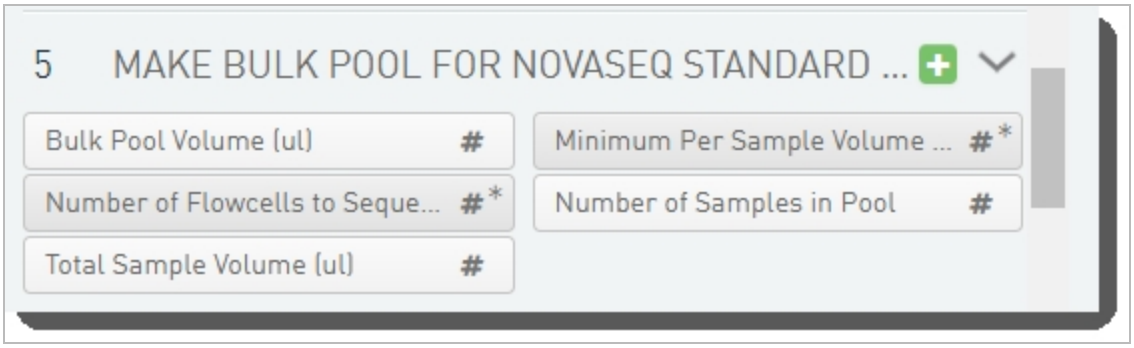
Automatically triggered on exit of the Record Details screen, this automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol - Dilute and Denature (NovaSeq 6000 v2.0):

```
nextStep = ::ADVANCE::
```

Step UDFs/custom fields

There are five fields defined on the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0) step.

These are shown below in the BaseSpace Clarity LIMS v5 Configuration area (Custom Fields > Master Step Fields tab). See Table 5 for LIMS v4.2 and LIMS v5 field configuration details.



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Table 5: Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0) step UDF / custom field configuration

Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
Bulk Pool Volume (ul) (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2
Number of Flowcells to Sequence	Numeric	• Required: Yes • Read Only: No	• Range From:1 to 10 • Decimal Places Displayed: 0
Total Sample Volume (ul) (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Default: 0 • Decimal Places Displayed: 2
Minimum Per Sample Volume (uL)	Numeric	• Required: Yes • Read Only: No	• Default: 5 • Decimal Places Displayed: 2
Number of Samples in Pool (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Default: 0 • Decimal Places Displayed: 0

Global UDFs/custom fields

Table 6 lists the global UDFs / custom fields that are configured to display on the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0) step.

Table 6: Global UDF / custom field configuration

Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
Configured on Analyte in LIMS v4.2 and Derived Samplein LIMS v5:			

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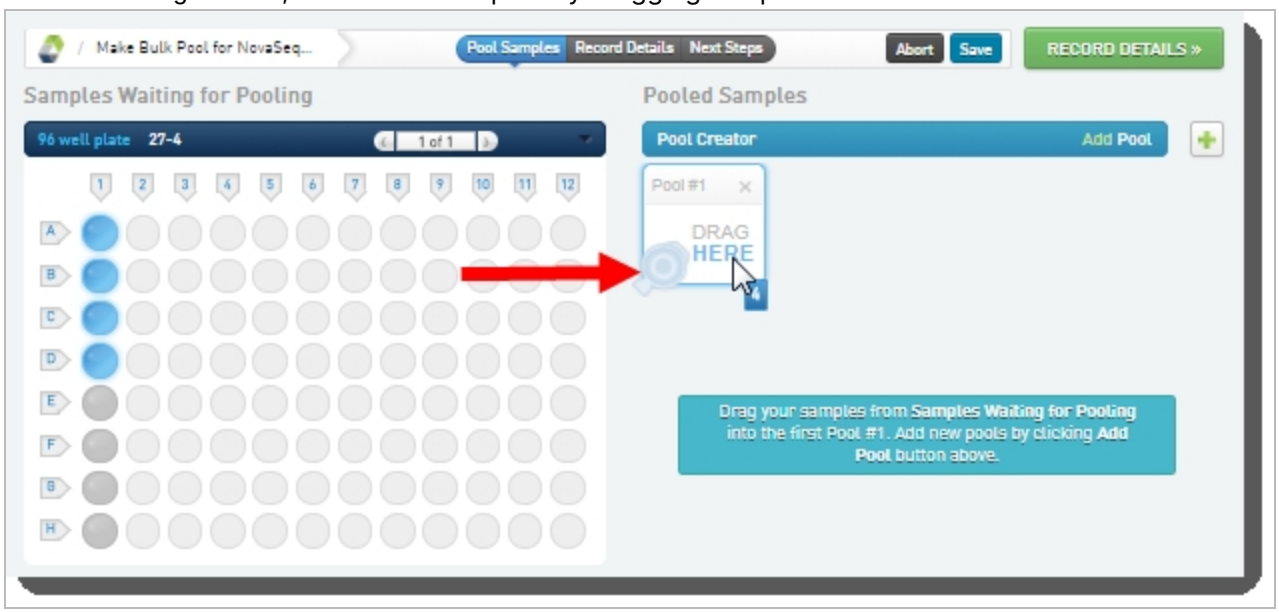
Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
Flowcell Type	Single-line Text (v4.2) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• S1 • S2 • S4
Loading Workflow Type	Single-line Text (v4.2) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
NaOH Volume (ul)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
RSB Volume (ul)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Tris-HCl Volume (ul)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Volume of Pool to Denature (ul) *Used in Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0) step only. Displays on Record Detailsscreen and in the generated CSV file.*	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0

User interaction

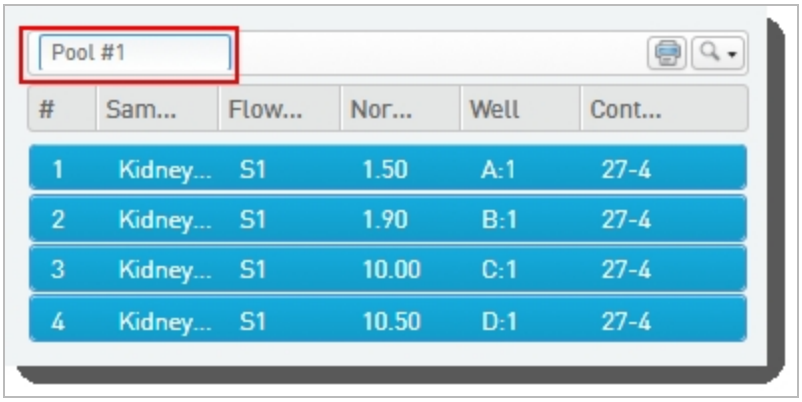
1. On the Queue screen, user adds samples of the same Flowcell Type to the Ice Bucket and clicks View Ice Bucket.
2. On the Ice Bucket screen, user clicks Begin Work.

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3. On the Pooling screen, user: Creates a pool by dragging samples into the Pool Creator.



- Names the pool, or accepts the default name - Pool #1.

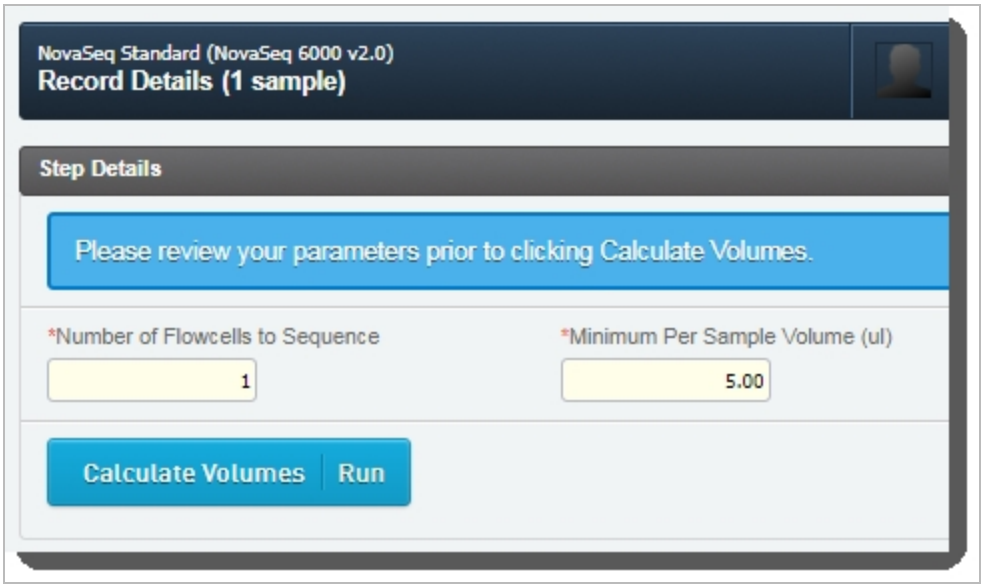


- Clicks Record DetailsOn exit from the Pooling screen, the Validate Inputs Flowcell Type and Single Pool is triggered. The automation checks that all samples in the pool have been assigned the same Flowcell Type, and only one pool has been created.

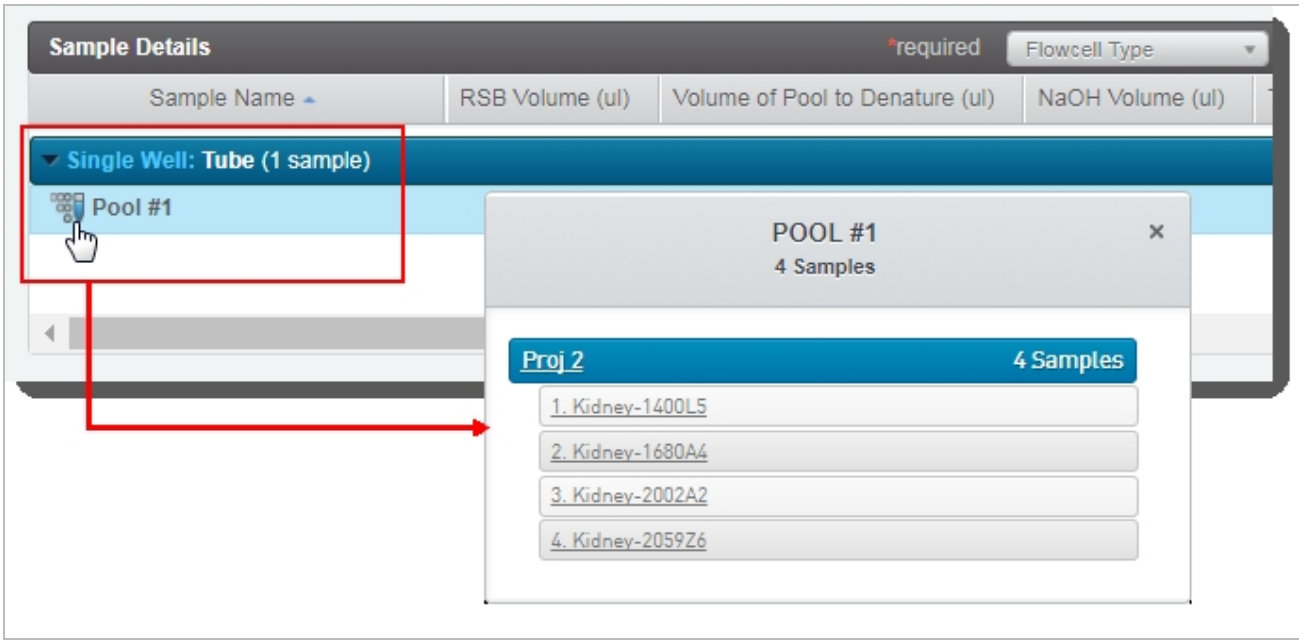
4. On the Record Details screen, the Step Details area contains two required fields:Number of Flowcells to Sequence: The value entered in this field is used in volume calculations, to ensure that volumes are sufficient for the number of times the pool will be sequenced.
    - Minimum Per Sample Volume (ul):In many applications, and particularly where robotics are used, a minimum sample volume is required. The value in this field is used to calculate how

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much of each sample will be included in the pool. The field is pre-populated with the configured default value, 5 ul, but may be edited if required.



- In the Sample Details table, the user can click on the pool icon to view details on the pool composition.



(Click image to view full size)

- The user clicks Calculate Volumes. This triggers the Calculate Volumes automation, which performs volume calculations based on the selected Flowcell Type : Calculates Bulk Pool Volume (ul) value.

– Calculates the Per Sample Volume (ul) value to be added to the pool.

– Calculates the Total Sample Volume (ul) value.

- If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (ul) value.
- Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
- Populates the Volume of Pool to Denature (ul), NaOH Volume (ul) and Tris-HCl Volume (ul) columns of the Sample Details table. (Values are set by a script and are not editable by the user.)
- Generates the Calculation File (CSV) and attaches it to the step. This file contains volume information about the pool and the individual samples it contains. The user can click on the file to download it, and then open it in Excel.
    - The user clicks Next Steps. This triggers the Set Next Step automation. This automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol - Dilute and Denature (NovaSeq 6000 v2.0).

5. On the Assign Next Steps screen: The next step is already set to Dilute and Denature (NovaSeq 6000 v2.0).
    - User clicks Finish Step. At the end of this step, the pool of samples automatically advances to the Dilute and Denature (NovaSeq 6000 v2.0) step.

Step 2: Dilute and Denature (NovaSeq 6000 v2.0)

In this step, pooled samples are denatured and diluted by the addition of NaOH, Tris-HCl, and Resuspension Buffer (RSB), and are manually placed by the user into the library tube that will be used in the NovaSeq run. In addition, this step generates the sample sheet file and the run recipe (JSON file) needed to start the NovaSeq run.

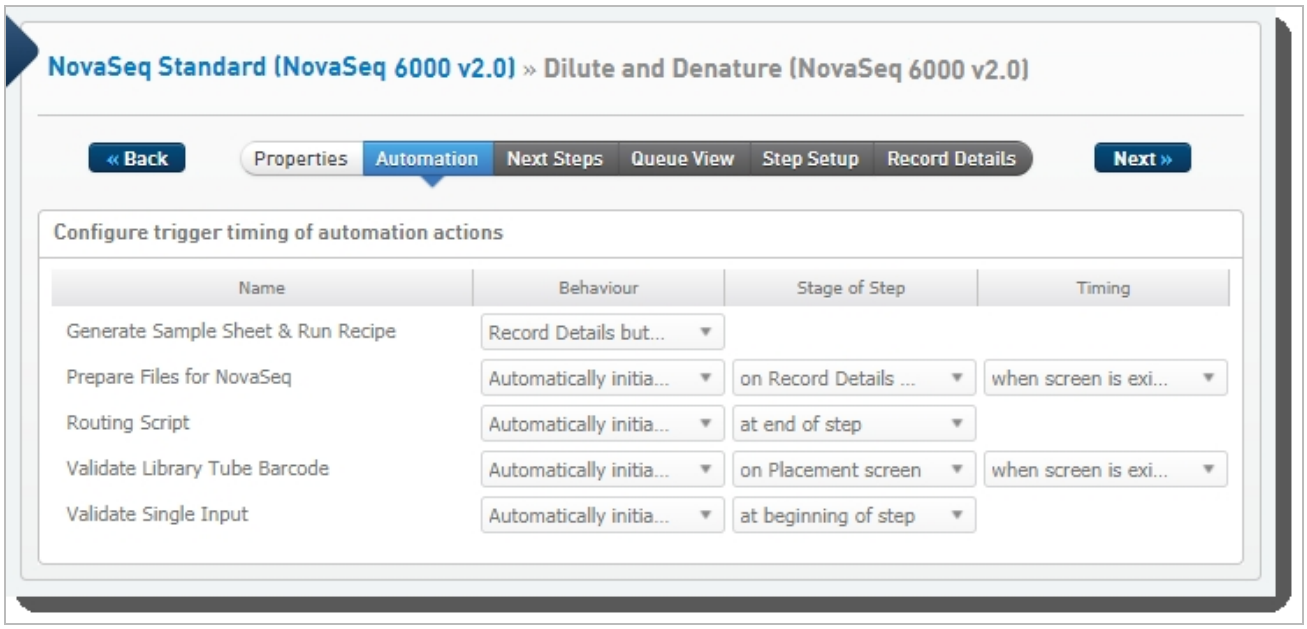
Step input: Bulk pool

Step output: Library tube

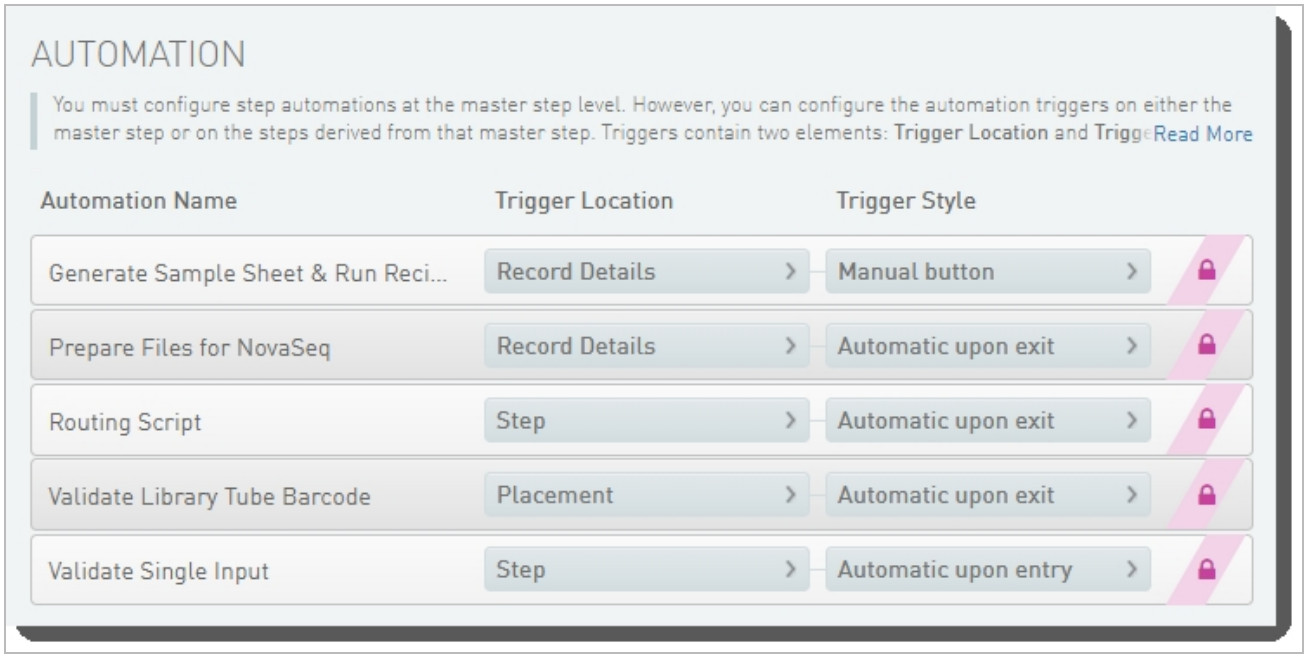
The following automations are configured on the step:

- Generate Sample Sheet and Run Recipe
- Prepare Files for NovaSeq
- Routing Script
- Validate Library Tube Barcode
- Validate Single Input

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Dilute and Denature (NovaSeq 6000 v2.0) automation configuration - LIMS v4.2



Dilute and Denature (NovaSeq 6000 v2.0) automation configuration - LIMS v5

These automations are described below, in the order in which they are triggered at run time.

Validate Single Input

Automatically triggered at the beginning of the step, this automation checks that there is only one container input to the step.

```
script:validateSampleCount -min 1 -max 1
```

Validate Library Tube Barcode

Automatically triggered on exit of the Placement screen, this automation validates the library tube barcode to ensure it conforms to the barcode mask [A-Za-z]{2}[0-9]{7}-[A-Za-z]{3}:

```
if (!output.container.name.matches(::[A-Za-z]{2}[0-9]{7}-[A-Za-z]{3}::)) { fail
(::Invalid Library Tube Barcode. Please verify and try again.::) }
```

Generate Sample Sheet and Run Recipe

Automatically triggered when user clicks a button on the Record Details screen, this automation:

- Copies the Flowcell Type from the step input to the step output:

```
output.::Flowcell Type:: = input.::Flowcell Type::
```

- Copies the Flowcell Type from the step input to the Run Mode field (hidden):

```
step.::Run Mode:: = input.::Flowcell Type::
```

- Copies the Loading Workflow Type values from the step inputs to the step outputs:

```
output.::Loading Workflow Type:: = input.::Loading Workflow Type::;
```

- Validates the parameters entered on the Record Details screen:
  - Experiment Name may only contain alphanumeric, dash, or underscore characters. Spaces are not permitted.
  - When Workflow Type = No Index, Index Read 1 must be zero. For any other Workflow Type, Index Read 1 must be greater than zero.

```
if(!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) { fail(::Experiment
Name contains prohibited characters. Allowed characters are: a-z, A-Z, 0-9, -,
and _::)};if(step.::Workflow Type::== ::No Index::) { if(step.::Index Read 1::!=
0) { fail(::Index Read 1 must be 0 if the Workflow Type is No Index.::) }} else {
if(step.::Index Read 1::== 0) { fail(::Index Read 1 must be greater than 0 if the
Workflow Type is ::+ step.::Workflow Type::+ ::.::) } };
```

- Sets Read 2 Cycles value:
  - If Paired End = True, copies the value of Read 1 Cycles into Read 2 Cycles.
  - If Paired End = False, sets Read 2 Cycles to 0.

```
step.::Read 2 Cycles:: = step.::Paired End::.toBoolean() ? step.::Read 1 Cycles::
: 0;
```

- Determines Index Read 2 based on the Workflow Type and Index Read 1: When Workflow Type = No Index or Single Index, Index Read 2 is set to 0.
  - When Workflow Type = Dual Index or Custom, Index Read 2 is set to the same as Index Read 1.

```
if (step.::Workflow Type:: == ::No Index:: || step.::Workflow Type:: == ::Single
Index::) { step.::Index Read 2:: = 0 } else { step.::Index Read 2:: =
step.::Index Read 1:: }
```

- Sets the next step for samples to REMOVE:

```
nextStep = ::REMOVE::
```

- Generates the sample sheet and the run recipe file and attaches them to the step. For details on how the files are generated, and their contents, see Sample sheet and run recipe file generation section, below.

Prepare Files for NovaSeq

Automatically triggered on exit of the Record Details screen, this automation invokes the copy_attachments_to_network_folder script, which is included in the novaseq-remote-extensions.jar file. This script places the generated sample sheet and run recipe (JSON file) into the appropriate folder on the NAS, where the instrument will use them to start the run.

For more information, see Sample sheet and run recipe file generation section, below.

Routing Script

Automatically triggered on exit of the step, this automation invokes the changeWorkflow script, which routes step outputs to the NovaSeq 6000 v2.0 workflow, and queues them for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) step.

Default automation command line:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -u {username} -p {password} -i {stepURI:v2} -l {compoundOutputFileLuid2} script:changeWorkflow \ \ --FIELD_NAME 'N/A' \ --FIELD_VALUE 'N/A' \ --WORKFLOW 'NovaSeq 6000 v2.0' \ --STEP 'AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS'"
```

Step UDFs/custom fields

There are 14 fields defined on the Dilute and Denature (NovaSeq 6000 v2.0) step. These fields are required for sample sheet and JSON file generation..

These fields are shown below in the BaseSpace Clarity LIMS v5 Configuration area (Custom Fields > Master Step Fields tab). See Table 7 for LIMS v4.2 and LIMS v5 field configuration details.

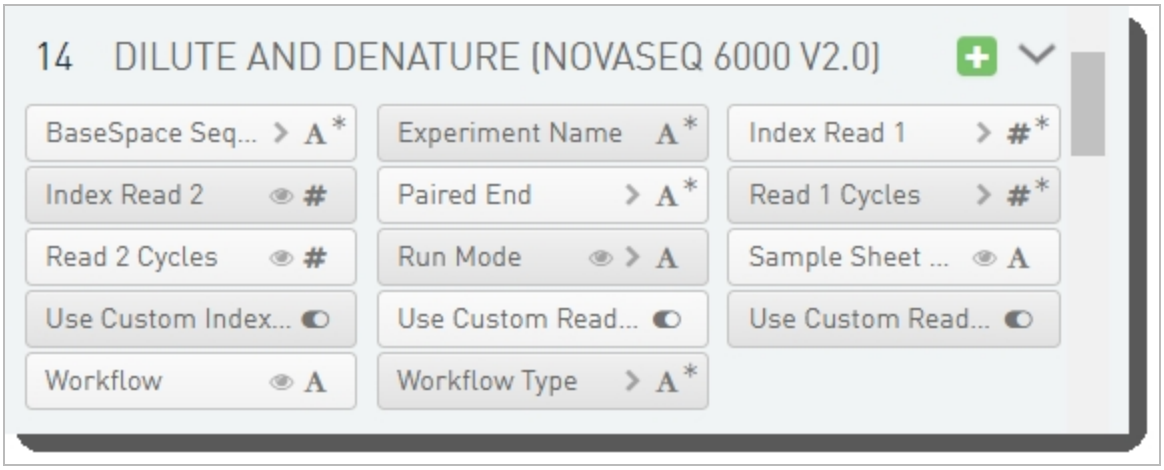


Table 7: Dilute and Denature (NovaSeq 6000 v2.0) step UDF / custom field configuration

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Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
BaseSpace Sequence Hub Configuration	Single-line Text (v4.2) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• Not Used • Run Monitoring Only • Run Monitoring and Storage
Experiment Name	Single-line Text (v4.2) Text (v5)	• Required: Yes • Read Only: No	
Index Read 1	Numeric (v4.2) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 0 • 6 • 8 • Range From: 0 to 20 • Decimal Places Displayed: 0
Index Read 2	Numeric (v4.2) Numeric (v5)	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0
Paired End	Single-line Text (v4.2) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• True • False
Read 1 Cycles	Numeric (v4.2) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 151 • 101 • 51 • Range From: 1 to 151 • Decimal Places Displayed: 0
Read 2 Cycles	Numeric (v4.2) Numeric (v5)	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0
Run Mode *(not displayed in user interface)*	Single-line Text (v4.2) Text Dropdown (v5)	• Required: No • Read Only: Yes • Custom Entries: No	• S1 • S2 • S4

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Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
Sample Sheet Path	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: Yes	
Use Custom Index Read 1 Primer *LIMS v5.0.5 and later: See Configuration Updatedocumentation.*	Check Box (v4.2) Toggle Switch (v5)	• Required: No • Read Only: No	• None Set
Use Custom Read 1 Primer *LIMS v5.0.5 and later: See Configuration Updatedocumentation.*	Check Box (v4.2) Toggle Switch (v5)	• Required: No • Read Only: No	• None Set
Use Custom Read 2 Primer *LIMS v5.0.5 and later: See Configuration Updatedocumentation.*	Check Box (v4.2) Toggle Switch (v5)	• Required: No • Read Only: No	• None Set
Workflow	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: Yes	GenerateFASTQ
Workflow Type	Single-line Text (v4.2) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• No Index • Single Index • Dual Index • Custom

Global UDFs/custom fields

Table 8 lists the global UDFs / custom fields that are configured to display on the Dilute and Denature (NovaSeq 6000 v2.0) step.

Table 8: Global UDF / custom field configuration

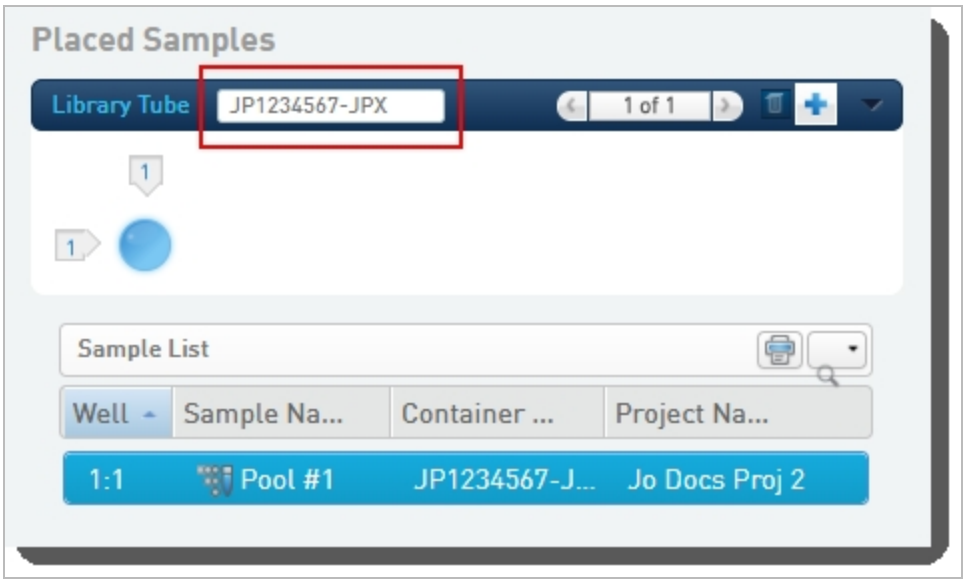
Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
Configured on Analyte in LIMS v4.2 and Derived Samplein LIMS v5:			
Flowcell Type	• Single-line Text (v4.2) • Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• S1 • S2 • S4

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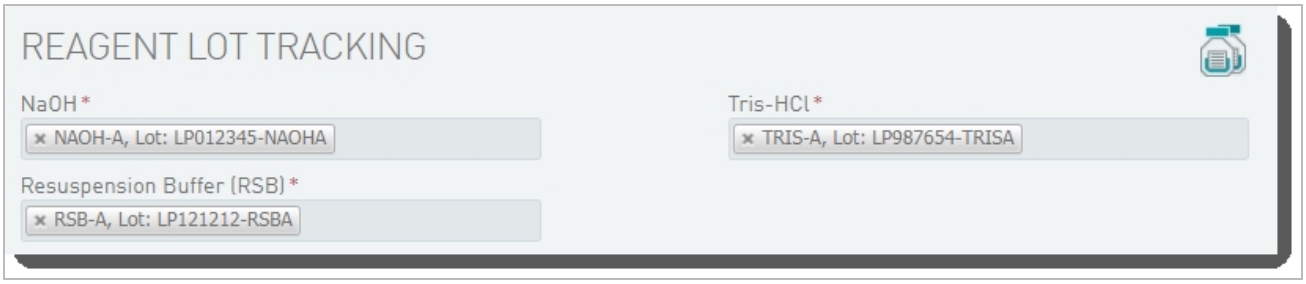
Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
Loading Workflow Type	• Single-line Text (v4.2) • Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

User interaction

1. On the Placement screen, user does the following: Drags the pool into the library tube in the Placed Samples area.
    - Scans or types the barcode of the library tube into the Library Tubefield.



    - Clicks Record Details.On exit of the Placement screen, the Validate Library Tube Barcode automation checks that the library tube barcode conforms to the barcode mask [A-Za-z]{2}[0-9]{7}-[A-Za-z]{3}, and displays an error message if this is not the case.
2. On the Record Details screen:
    - The Reagent Lot Tracking section lets you track the NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. The user selects from the active lots displayed in each drop-down list.



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Note:
To make reagent lots available for selection in the Reagent Lot Tracking section, you must first configure and activate them. You can do this on the Reagents and Controls screen (LIMS v4.2) or the Configuration > Consumables screen (LIMS v5).

- The fields displayed in the Step Details section are used to generate the sample sheet and run recipe files. Some of these fields are auto-populated and some must be completed by the user (see Table 9, below, for details). *Table 9: Fields displayed on Record Details screen of Dilute and Denature (NovaSeq 6000 v2.0) step*

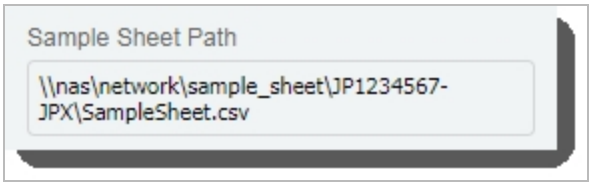
Field	Value
Experiment Name	User enters the name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.
BaseSpace Sequence Hub Configuration	User selects from preset options: Not Used, Run Monitoring Only, or Run Monitoring and Storage
Workflow	Automatically populated with preset - GenerateFASTQ.
Workflow Type	User selects from preset options: No Index, Single Index, Dual Index, or Custom
Index Read 1	User selects from preset option: 0, 6, or 8 - or types a value between 0 and 20
Index Read 2	If user selected the Dual Index workflow type, once the sample sheet has been generated, Index Read 2 value is automatically populated to match Index Read 1.
Paired End	User selects from preset options: True or False
Read 1 Cycles	User selects from preset options: 151, 101, or 51 - or types a value between 1 and 151
Read 2 Cycles	If user selected Paired End =True, once the sample sheet has been generated, Read 2 Cycles value is automatically populated to match Read 1 Cycles.
Use Custom Read 1 Primer	User selects if applicable.
Use Custom Read 2 Primer	User selects if applicable.
Use Custom Index Read 1 Primer	User selects if applicable.
Sample Sheet Path	Automatically populated once the sample sheet has been generated.
Workflow	Automatically populated with preset - GenerateFASTQ.

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- User clicks Generate Sample Sheet and Run Recipe. This triggers the automation script, which does the following.
- Generates the sample sheet and JSON files and attaches them to the placeholders in the Files area of the Record Detailsscreen.



- Populates the Sample Sheet Pathfield with the path to the sample sheet file.



- If applicable, populates the Index Read 2 and Read 2 Cycles fields.
- Sets the value of the next step to Remove from workflow. The Routing Script automation expects this value, and requires it in order to successfully advance samples to the next step.
- User clicks Next Steps. This triggers the Prepare Files for NovaSeq automation, which copies the sample sheet and run recipe files to the location specified during installation.

3. On the Assign Next Steps screen:
    - The Next Step for all samples is pre-populated with Remove from workflow.

    The user must not change this value! If Next Step is not set to Remove from workflow, the routing script will not be able to route samples correctly.

    - User clicks Finish Step.
4. On exit from the step: The Routing Script automation is triggered and samples are routed to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) protocol.
    - In Lab View, the pool of samples is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) step.

At this point in the workflow, the user interaction ends. The integration automatically starts the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) step and data from the run is parsed back into the LIMS.

Protocol 3: NovaSeq Xp (NovaSeq 6000 v2.0)

Samples are routed to this protocol if their Loading Workflow Type value is set to NovaSeq Xp.

Samples are pooled and added to lanes on the NovaSeq flow cell type selected in the Define Run Format (NovaSeq 6000 v2.0) step. Resuspension buffer is added.

At the end of the protocol, a routing script sends the flow cell to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) protocol.

This protocol contains three steps, described below:

- Step 1: Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.0)
- Step 2: Dilute, Denature and ExAmp (NovaSeq 6000 v2.0)
- Step 3: Load to Flowcell (NovaSeq 6000 v2.0)

Step 1: Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.0)

In this step, user manually places libraries into a pool.

Note:

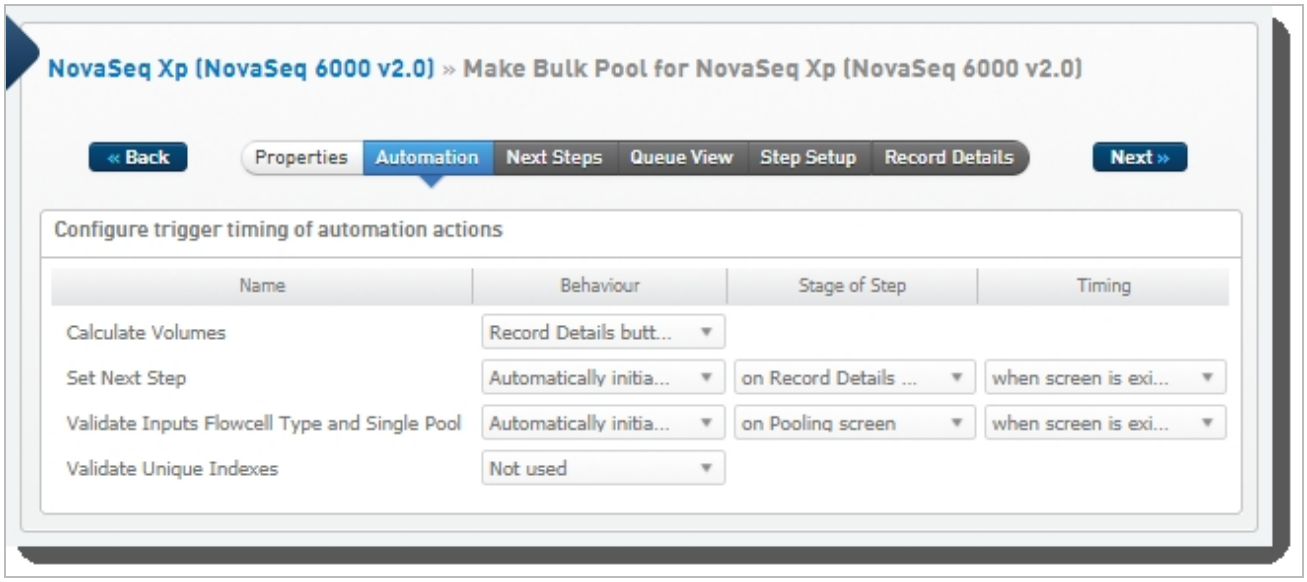
User must only create only one pool per step.

Step input: NTP (normalized libraries)

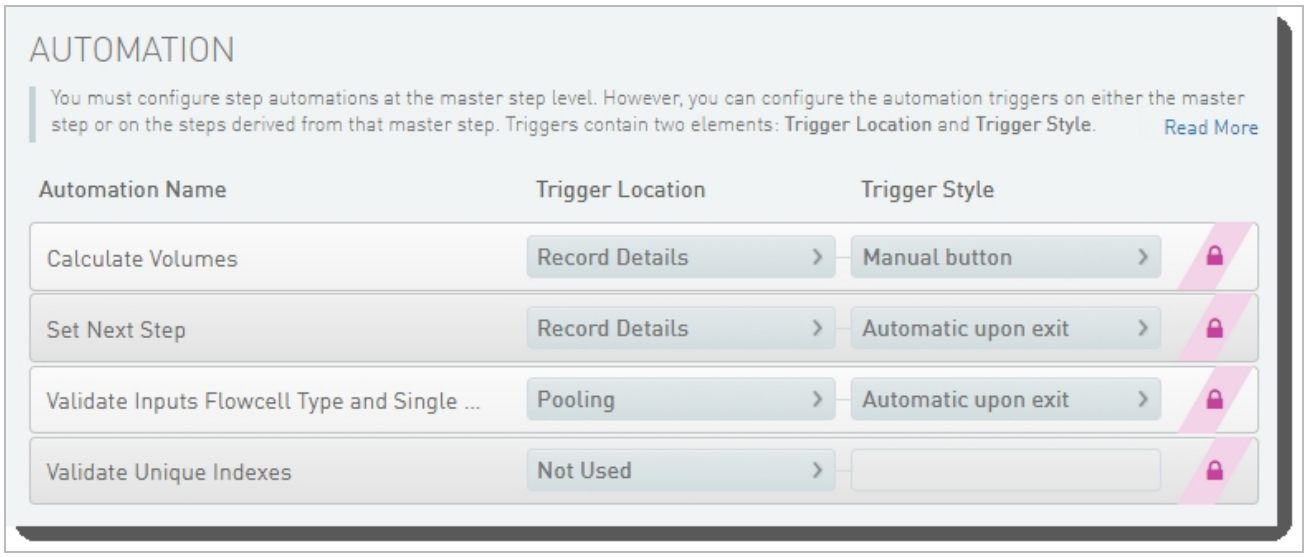
Step output: Bulk pool

The following automations are configured on the step:

- Calculate Volumes
- Set Next Step
- Validate Inputs Flowcell Type and Single Pool
- Validate Unique Indexes*(Not used. This functionality is handled by the core LIMS configuration for pooling.)*



Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.0) automation configuration - LIMS v4.2



Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.0) automation configuration - LIMS v5

These automations are described below, in the order in which they are triggered at run time.

Validate Inputs Flowcell Type and Single Pool

Automatically triggered on exit of the Pooling screen, this automation checks that:

- All samples in the pool have the same Flowcell Type assigned to them.
- Only one pool has been created.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/novaseq/novaseq-extensions.jar script:validate_flowcell_for_input_pools -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -validateSingleOutput true
```

Calculate Volumes

Automatically triggered when the user clicks the Calculate Volumes button on the Record Details screen, this automation:

- Calculates the number of samples in the pool:

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: + 1
```

- Sets the value of the Bulk Pool Volume (ul) field, based on the selected Flowcell Type:

```
if (input.::Flowcell Type:: == ::S1::) { step.::Bulk Pool Volume (ul):: = step.::Number of Lanes to Sequence:: * 18 + 30}; if (input.::Flowcell Type:: == ::S2::) { step.::Bulk Pool Volume (ul):: = step.::Number of Lanes to Sequence;; * 22 + 30};if (input.::Flowcell Type:: == ::S4::) { step.::Bulk Pool Volume (ul):: = step.::Number of Lanes to Sequence:: * 30 + 30};
```

- Calculates the Per Sample Volume (ul) to be added to the pool:

```
input.::Per Sample Volume (ul)::= (((input.::Final Loading Concentration (pM)::* 5/ 1000) / input.::Normalized Molarity (nM)::) * step.::Bulk Pool Volume (ul)::) / step.::Number of Samples in Pool::
```


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Note:
To ensure accurate pipetting of each sample in a pool for sequencing, the Per Sample Volume (ul) value must be equal to or higher than the Minimum Per Sample Volume (ul) (default value set at 5, may be edited by the user).
Assuming the default Minimum Per Sample Volume (ul) value of 5, for a given batch:
1. If the smallest Per Sample Volume (ul) value is less than 5, the LIMS automatically assigns a value of 5 to the sample's Adjusted Per Sample Volume (ul) field.
2. The LIMS then adjusts the Adjusted Per Sample Volume (ul) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

- Calculates the Total Sample Volume (ul) field value:

```
step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: +
input.::Adjusted Per Sample Volume (ul)::
```

- If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (ul) field value:

```
if (step.::Total Sample Volume (ul):: >= step.::Bulk Pool Volume (ul)::)
{output.::RSB Volume (ul):: = 0} else {output.::RSB Volume (ul):: = step.::Bulk
Pool Volume (ul):: - step.::Total Sample Volume (ul)::} ;
```

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs:

```
output.::Flowcell Type::= input.::Flowcell Type::;output.::Loading Workflow
Type::= input.::Loading Workflow Type::;
```

- Uses the NovaSeq_Xp_Bulk_Pool.csv and NovaSeq_Xp_Bulk_Pool2.csv template files to generate a single CSV file containing information about the bulk pool and the samples it contains. The generated file is available for download by the user on the Step Setup screen of the following step - Dilute, Denature and ExAmp (NovaSeq 6000 v2.0).

Set Next Step

Automatically triggered on exit of the Record Details screen, this automation:

- Copies the Flowcell Type values from the step inputs to the step outputs.
- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ; nextStep = ::ADVANCE::
```

Step UDFs/custom fields

There are five fields defined on the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.0) step.

These are shown below in the BaseSpace Clarity LIMS v5 Configuration area (Custom Fields > Master Step Fields tab). See Table 10 for LIMS v4.2 and LIMS v5 field configuration details.

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Table 10: Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.0) step UDF / custom field configuration

Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
Bulk Pool Volume (ul)*(for calculation purposes, not displayed)*	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2
Number of Lanes to Sequence	Numeric	• Required: Yes • Read Only: No	• Decimal Places Displayed: 0
Total Sample Volume (ul)*(for calculation purposes, not displayed)*	Numeric	• Required: No • Read Only: No	• Default: 0 • Decimal Places Displayed: 0
Minimum Per Sample Volume (uL)	Numeric	• Required: Yes • Read Only: No	• Default: 5 • Decimal Places Displayed: 2
Number of Samples in Pool*(for calculation purposes, not displayed)*	Numeric	• Required: No • Read Only: No	• Default: 0 • Decimal Places Displayed: 0

Global UDFs/custom fields

Table 11 lists the global UDFs / custom fields that are configured to display on the *Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.0)* step.

Table 11: Global UDF / custom field configuration

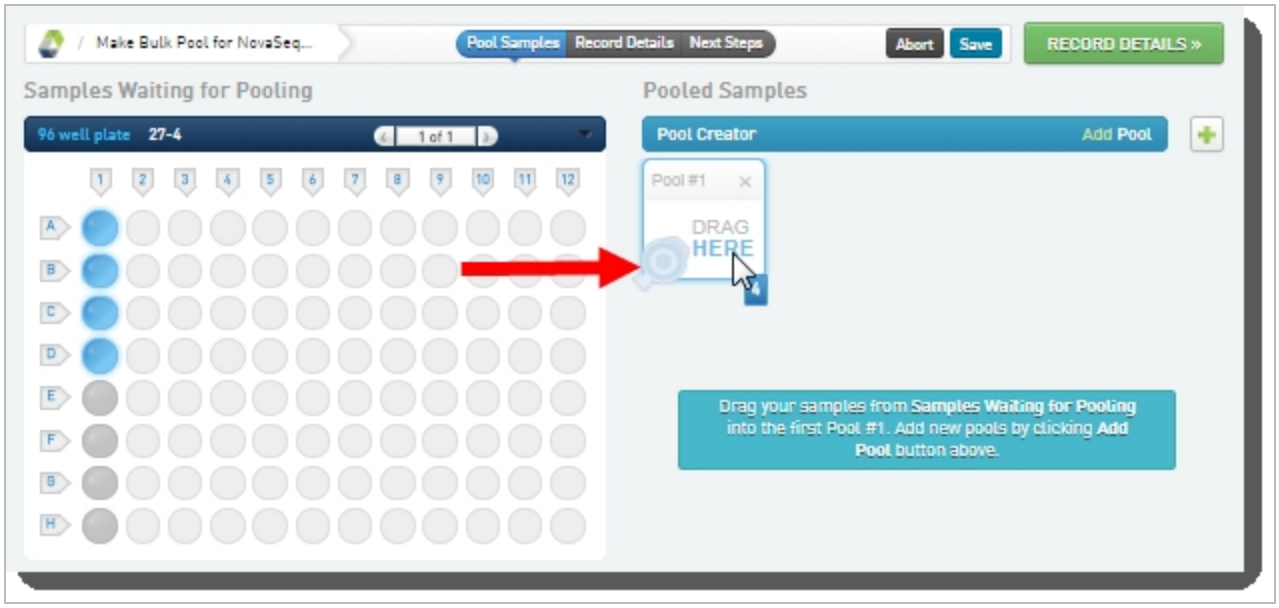
Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
RSB Volume (ul)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2

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Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
Flowcell Type	Single-line Text (v4.2) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• S1 • S2 • S4
Loading Workflow Type	Single-line Text (v4.2) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

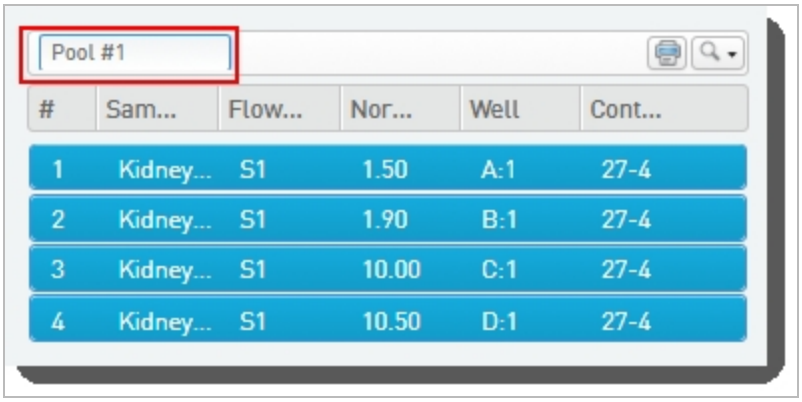
User interaction

1. On the Queue screen, user adds samples of the same Flowcell Type to the Ice Bucket and clicks View Ice Bucket.
2. On the Ice Bucket screen, user clicks Begin Work.
3. On the Pooling screen, user: Drags samples over into the Pool Creatorto create a pool.



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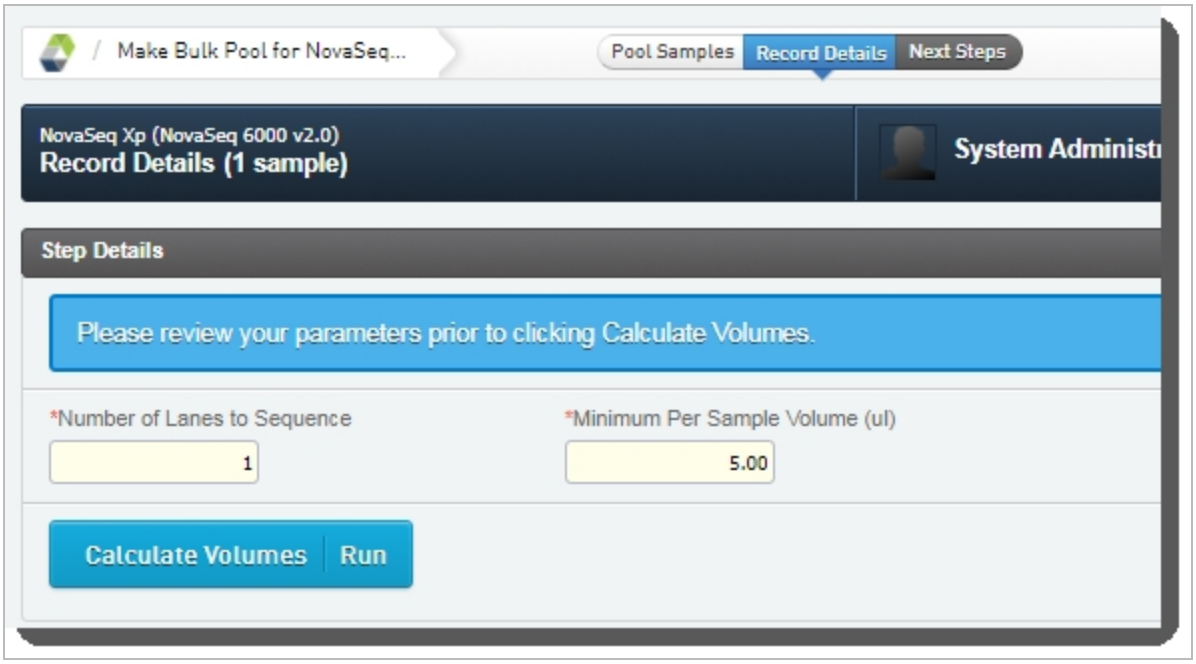
- Names the pool, or accepts the default name - Pool #1.



- Clicks Record Details.

On exit from the Pooling screen, the Validate Inputs Flowcell Type and Single Pool is triggered. The automation checks that all samples in the pool have been assigned the same Flowcell Type, and only one pool has been created.

4. On the Record Details screen, the Step Details area contains two required fields:
   - Number of Lanes to Sequence: The value entered in this field is used in volume calculations, to ensure that volumes are sufficient for the number of times the pool will be sequenced.
   - Minimum Per Sample Volume (ul):In many applications, and particularly where robotics are used, a minimum sample volume is required. The value in this field is used to calculate how much of each sample will be included in the pool. The field is pre-populated with the configured default value, 5 ul, but may be edited if required.



- In the Sample Details table, the user can click on the pool icon to view details on the pool composition.
- The user clicks Calculate Volumes. This triggers the Calculate Volumes automation, which performs volume calculations based on the selected Flowcell Type:

– Calculates Bulk Pool Volume (ul) value.
– Calculates the Per Sample Volume (ul) value to be added to the pool.
– Calculates the Total Sample Volume (ul) value.
– If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (ul) value.
– Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
– Generates the Calculation File (CSV) and attaches it to the step. This file contains volume information about the samples and RSB buffer to add to the pool. The user can click on the file to download it, and then open it in Excel.

- The user clicks Next Steps. This triggers the Set Next Step automation. This automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol - Dilute, Denature and ExAmp (NovaSeq 6000 v2.0.

5. On the Assign Next Steps screen:
    - The next step is already set to Dilute, Denature and ExAmp (NovaSeq 6000 v2.0).
    - User clicks Finish Step. At the end of this step, the pool of samples automatically advances to the Dilute, Denature and ExAmp (NovaSeq 6000 v2.0) step.

Step 2: Dilute, Denature and ExAmp (NovaSeq 6000 v2.0)

In this step, pooled samples are denatured and diluted by the addition of DPX, NaOH, Tris-HCl, and RSB. The user manually creates working pools based on the number of lanes they want to sequence.

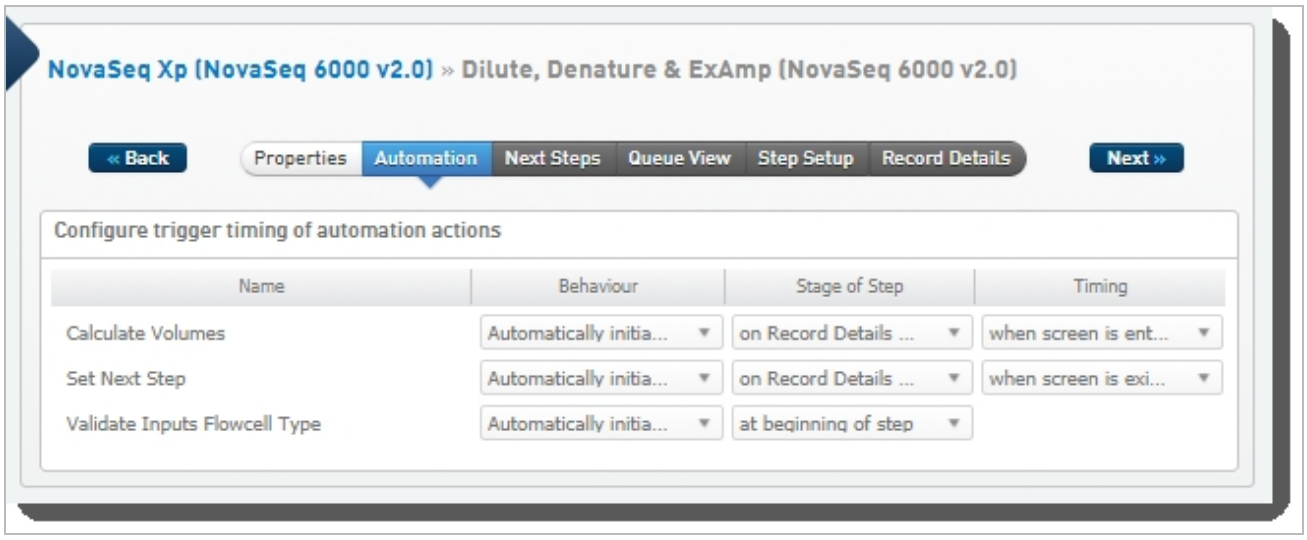
Step input: Bulk pool

Step output: Working pool - variable number, the user chooses how many working pools to create per bulk pool

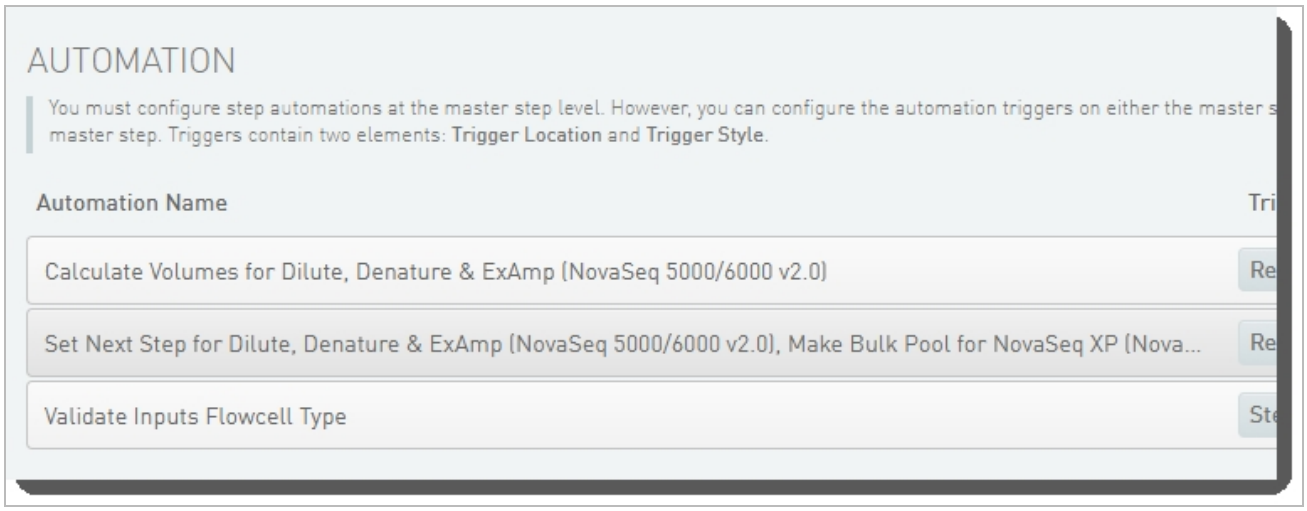
The following automations are configured on the step:

- Calculate Volumes
- Set Next Step
- Validate Inputs Flowcell Type

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Dilute, Denature and ExAmp (NovaSeq 6000 v2.0) automation configuration - LIMS v4.2



Dilute, Denature and ExAmp (NovaSeq 6000 v2.0) automation configuration - LIMS v5

These automations are described below, in the order in which they are triggered at run time.

Validate Inputs Flowcell Type

Automatically triggered at the beginning of the step, this automation invokes the validate_flowcell_for_input_pools script. This script does the following:

- Checks the inputs to the step and validates that the Flowcell Type field has been set to a valid value (S1, S2, or S4), and that all inputs have the same value.
- Validates that the number of outputs matches the number of lanes on the selected flow cell type. If validation fails, an error message informs the user that the number of working pools does not match the number of lanes available on the flow cell.

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```
bash -l -c "/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/novaseq/novaseq-extensions.jar script:validate_
flowcell_for_input_pools -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1}"
```

Calculate Volumes

Automatically triggered on entry to of the Record Details screen, this automation sets the value of the following fields, based on the Flowcell Type:

- BP Aliquot Volume (ul)
- NaOH Volume (ul)
- Tris-HCl Volume (ul)
- DPX1 Volume (ul)
- DPX2 Volume (ul)
- DPX3 Volume (ul)
- Mastermix per Lane (ul)

```
if (input.::Flowcell Type:: == ::S1::) {  output.::BP Aliquot Volume (ul):: = 18;
output.::NaOH Volume (ul):: = 4.50;  output.::Tris-HCl Volume (ul):: = 4.50;
step.::DPX1 Volume (ul):: = 126;  step.::DPX2 Volume (ul):: = 18;  step.::DPX3
Volume (ul):: = 66;  output.::Mastermix per Lane (ul):: = 63 }; if
(input.::Flowcell Type:: == ::S2::) {  output.::BP Aliquot Volume (ul):: = 22;
output.::NaOH Volume (ul):: = 5.50;  output.::Tris-HCl Volume (ul):: = 5.50;
step.::DPX1 Volume (ul):: = 126;  step.::DPX2 Volume (ul):: = 18;  step.::DPX3
Volume (ul):: = 66;  output.::Mastermix per Lane (ul):: = 77 }; if
(input.::Flowcell Type:: == ::S4::) {  output.::BP Aliquot Volume (ul):: = 30;
output.::NaOH Volume (ul):: = 7.50;  output.::Tris-HCl Volume (ul):: = 7.50;
step.::DPX1 Volume (ul):: = 315;  step.::DPX2 Volume (ul):: = 45;  step.::DPX3
Volume (ul):: = 165;  output.::Mastermix per Lane (ul):: = 105 };
```

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs:

```
output.::Flowcell Type::= input.::Flowcell Type::;output.::Loading Workflow
Type::= input.::Loading Workflow Type::;
```

- Uses the NovaSeq_Xp_Working_Pool.csv and NovaSeq_Xp_Working_Pool2.csv template files to generate a single CSV file containing information about the DPX volume, and the volume of BP Aliquot, Mastermix, NaOH, and Tris-HCl to add per working pool. The generated file is available for download by the user on the Step Setup screen of the following step - Load to Flowcell (NovaSeq 6000 v2.0).

Set Next Step

Automatically triggered on exit of the Record Details screen, this automation:

- Copies the Flowcell Type values from the step inputs to the step outputs.

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- Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol:

```
output.::Flowcell Type:: = input.::Flowcell Type:: ; nextStep = ::ADVANCE::
```

Step UDFs/custom fields

There are 3 fields defined on the Dilute, Denature and ExAmp (NovaSeq 6000 v2.0) step. (These field values are set by a script and are not editable by the user running the step.)

The fields are shown below in the BaseSpace Clarity LIMS v5 Configuration area (Custom Fields > Master Step Fields tab). See Table 12 for LIMS v4.2 and LIMS v5 field configuration details.

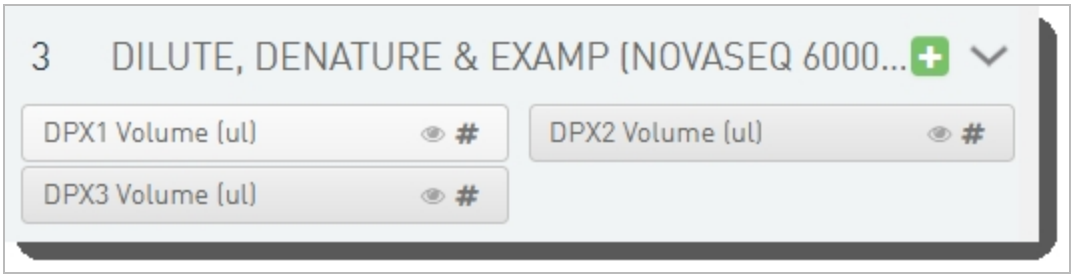


Table 12: Dilute, Denature and ExAmp (NovaSeq 6000 v2.0) step UDF / custom field configuration

Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
DPX1 Volume (ul)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0
DPX2 Volume (ul)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0
DPX3 Volume (ul)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0

Global UDFs/custom fields

Table 13 lists the global UDFs / custom fields that are configured to display on the Dilute, Denature and ExAmp (NovaSeq 6000 v2.0) step.

Table 13: Global UDF / custom field configuration

Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
Configured on Analyte in LIMS v4.2 and Derived Samplein LIMS v5:			
BP Aliquot Volume (ul)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0
Flowcell Type	Single-line Text (v4.2) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• S1 • S2 • S4

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Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
Loading Workflow Type	Single-line Text (v4.2) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
Mastermix per Lane (ul)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0
NaOH Volume (ul)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Tris-HCl Volume (ul)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2

User interaction

1. On the Queue screen, user adds the pool to the Ice Bucket and clicks View Ice Bucket.
2. On the Ice Bucket screen, user sets the number of derivatives to create (these will be placed into the flow cell lanes) and clicks Begin Work. On entry to the Record Details screen, the Calculate Volumes automation is triggered. This automation sets the following values based on the selected Flowcell Type (calculation details are provided inCalculate Volumes automation section, above):
    - BP Aliquot Volume (ul)
    - NaOH Volume (ul)
    - Tris-HCl Volume (ul)
    - DPX1 Volume (ul)
    - DPX2 Volume (ul)
    - DPX3 Volume (ul)
    - Mastermix per Lane (ul)
    - The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains information about the DPX Mastermix volume, and the volume of Mastermix, NaOH. and Tris-HCl to add per working pool. The user can click on the file to download it and then open it in Excel.
3. On the Record Details screen:
    - The Reagent Lot Tracking section lets you track the DPX1, DPX2, DPX3, NaOH, RSB, and Tris-HCl reagent lots. The user selects from the active lots displayed in each drop-down list.



Note:
To make reagent lots available for selection in the Reagent Lot Tracking section, you must first configure and activate them. You can do this on the Reagents and Controls screen (LIMS v4.2) or the Configuration > Consumables screen (LIMS v5).

- In the Step Details area, the DPX1, DPX2, and DPX3reagent volume values are already populated. (These values are set by a script and are not editable by the user running the step.)



- In the Sample Details table: The user can click on the pool icon to view details on the working pool composition.

– The BP Aliquot, Mastermix per lane, NaOH, and Tris-HCI volume values for each working pool are already populated. (These values are set by a script and are not editable by the user running the step.)
– The working pool number is appended to the bulk pool name. This allows users to quickly identify which working pools are derived from the same bulk pool.
  - In the Files area, the user can click on the Calculation File (CSV) file to download it and then open it in Excel to view information about the DPX Mastermix volume, and the volume of Mastermix, NaOH, and Tris-HCI to add per working pool.
  - User clicks Next Steps, which triggers the Set Next Step automation (described above).

4. On the Assign Next Steps screen, the next step is already set to Load to Flowcell (NovaSeq 6000 v2.0). User clicks Finish Step.

Step 3: Load to Flowcell (NovaSeq 6000 v2.0)

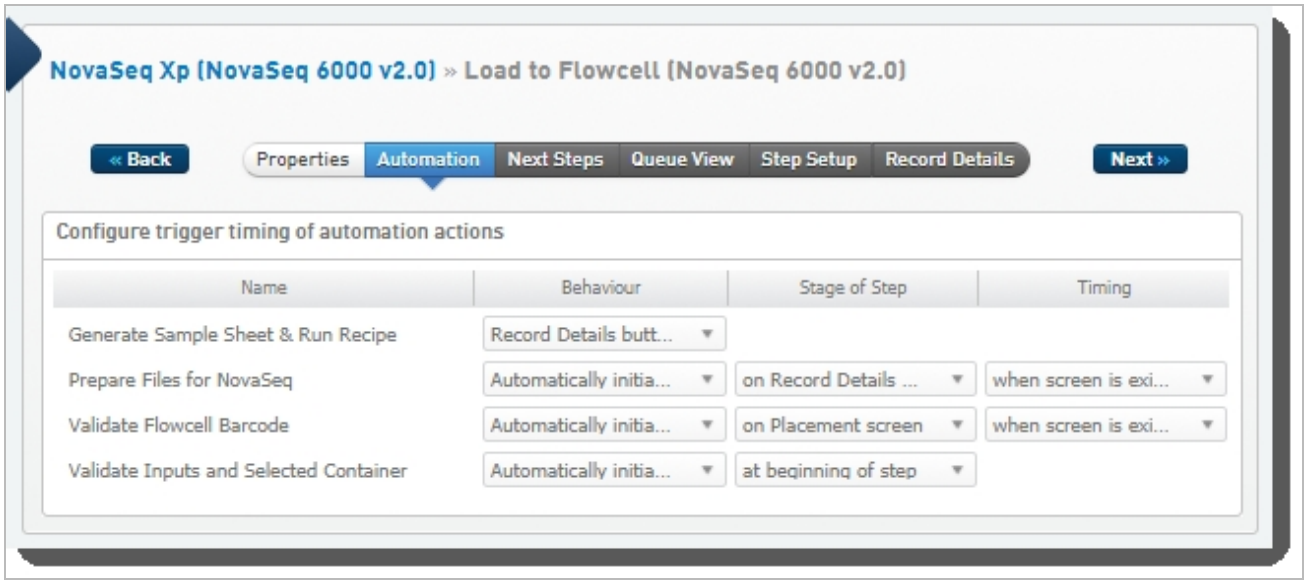
In this step, the user scans the flow cell barcode into the LIMS, and then manually places the working pools into the lanes of the flow cell that will be used in the NovaSeq run.

Step input: Working pool

Step output: Flow cell (output containers: S1 and S2 with 2 lanes, and S4 with 4 lanes)

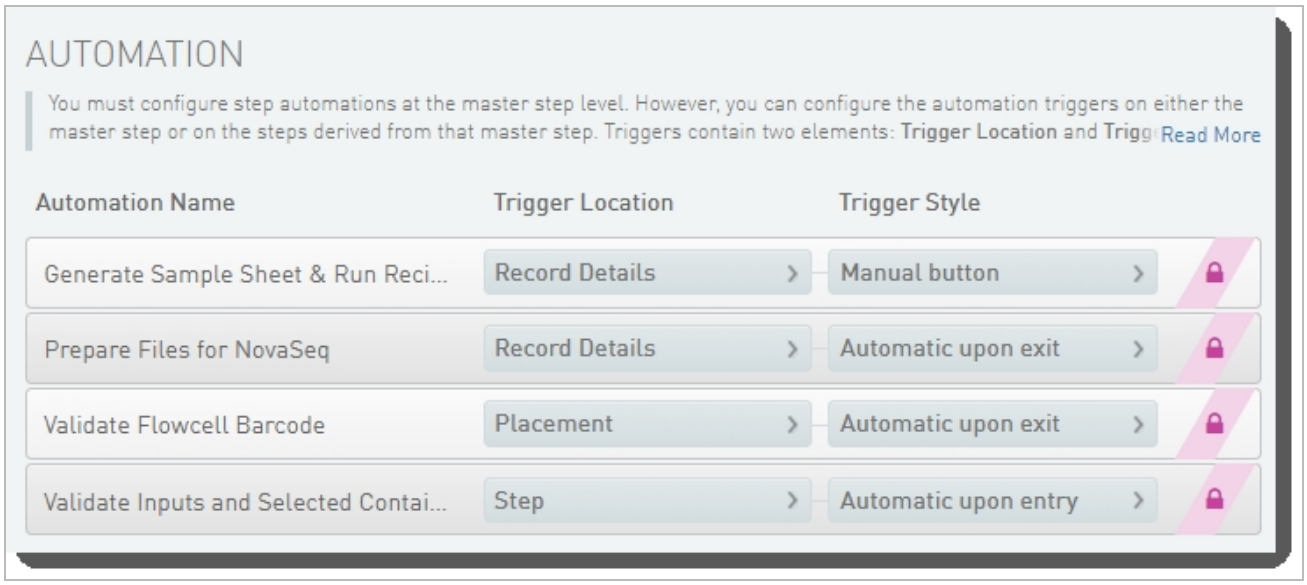
The following automations are configured on the step:

- Generate Sample Sheet and Run Recipe
- Prepare Files for NovaSeq
- Validate Flowcell Barcode
- Validate Inputs and Selected Container



Load to Flowcell (NovaSeq 6000 v2.0) automation configuration - LIMS v4.2

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Load to Flowcell (NovaSeq 6000 v2.0) automation configuration - LIMS v5

These automations are described below, in the order in which they are triggered at run time.

Validate Inputs and Selected Container

Automatically triggered at the beginning of the step, this automation invokes the validate_flowcell_for_input_pools and validateSelectedContainer scripts. These scripts validate the step inputs and the container selected by the user, as follows:

- Check that the Flowcell Type field has been set to a valid value (S1, S2, or S4), and that all inputs have the same value.
- Check that the number of outputs matches the number of lanes on the selected flow cell type. If validation fails, an error message informs the user that the number of working pools does not match the number of lanes available on the flow cell.
  The following table defines the number of samples allowed:

Flowcell Type	Number of Working Pools
S1	2
S2	2
S4	4

- Check that the the container type selected on entry to the Placement screen matches the value in the Flowcell Type field. If validation fails, displays an error message.

Validate Flowcell Barcode

Automatically triggered on exit of the Placement screen, this automation validates the flow cell barcode scanned into the LIMS by the user, using the following logic:

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```
if (input.::Flowcell Type:: == ::S1:: && !output.container.name.matches( ::[A-Za-z0-9]{5}DRXX:: ) ) {   fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: )   } ;   if (input.::Flowcell Type:: == ::S2:: && !output.container.name.matches( ::[A-Za-z0-9]{5}DMXX:: ) ) {   fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: )   } ;   if (input.::Flowcell Type:: == ::S4:: && !output.container.name.matches( ::[A-Za-z0-9]{5}DSXX:: ) ) { fail ( ::Invalid Flowcell Barcode. Please verify and try again.:: )   } ;
```

Generate Sample Sheet and Run Recipe

Automatically triggered when user clicks a button on the Record Details screen, this automation:

- Copies the Flowcell Type from the step input to the Run Mode field (hidden):

```
step.::Run Mode:: = input.::Flowcell Type::
```

- Validates the parameters entered on the Record Details screen:
  - Experiment Name may only contain alphanumeric, dash, or underscore characters. Spaces are not permitted.
  - When Workflow Type = No Index, Index Read 1 must be zero. For any other Workflow Type, Index Read 1 must be greater than zero.

```
if(!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) {  fail(::Experiment Name contains prohibited characters. Allowed characters are: a-z, A-Z, 0-9, -, and _::)};if(step.::Workflow Type::== ::No Index::) {  if(step.::Index Read 1::!= 0) {   fail(::Index Read 1 must be 0 if the Workflow Type is No Index.::) }} else{   if(step.::Index Read 1::== 0) {   fail(::Index Read 1 must be greater than 0 if the Workflow Type is ::+ step.::Workflow Type::+ ::.::)   } };
```

  - Sets Read 2 Cycles value:
  - If Paired End = True, copies the value of Read 1 Cycles into Read 2 Cycles.
  - If Paired End = False, sets Read 2 Cycles to 0.

```
step.::Read 2 Cycles:: = step.::Paired End::.toBoolean() ? step.::Read 1 Cycles:: : 0;
```

  - Determines Index Read 2 based on the Workflow Type and Index Read 1: When Workflow Type = No Index or Single Index, Index Read 2 is set to 0.
  - When Workflow Type = Dual Index or Custom, Index Read 2 is set to the same value as Index Read 1.

```
if (step.::Workflow Type:: == ::No Index:: || step.::Workflow Type:: == ::Single Index::) { step.::Index Read 2:: = 0 } else { step.::Index Read 2:: = step.::Index Read 1:: }
```

  - Copies the Flowcell Type value from the step inputs to the step outputs:

```
output.::Flowcell Type::= input.::Flowcell Type::;
```

  - Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol - AUTOMATED - Run (NovaSeq 6000) v2.0.

    nextStep = ::ADVANCE::

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- Generates the sample sheet and the run recipe file and attaches them to the step. For details on how the files are generated, and their contents, see Sample sheet and run recipe file generation section, below.

Prepare Files for NovaSeq

Automatically triggered on exit of the Record Details screen, this automation invokes the copy_attachments_to_network_folder script, which is included in the novaseq-remote-extensions.jar file. This script places the generated sample sheet and run recipe (JSON file) into the appropriate folder on the NAS, where the instrument will use them to start the run.

For more information, see Sample sheet and run recipe file generation section, below.

Step UDFs/custom fields

There are 15 fields defined on the Load to Flowcell (NovaSeq 6000 v2.0) step.

These are shown below in the BaseSpace Clarity LIMS v5 Configuration area (C ustom Fields > Master Step Fields tab). See Table 14 for LIMS v4.2 and LIMS v5 field configuration details.



Table 14: Load to Flowcell (NovaSeq 6000 v2.0) step UDF / custom field configuration

Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
BaseSpace Sequence Hub Configuration	Single-line Text (v4.2) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• Not Used • Run Monitoring Only • Run Monitoring and Storage
Experiment Name	Single-line Text (v4.2) Text (v5)	• Required: Yes • Read Only: No	

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Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
Index Read 1	Numeric (v4.2) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 0 • 6 • 8 • Range From: 0 to 20 • Decimal Places Displayed: 0
Index Read 2	Numeric (v4.2) Numeric (v5)	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0
Library Tube Barcode	Numeric (v4.2) Numeric (v5)	• Required: Yes • Read Only: No	• Decimal Places Displayed: 0
Paired End	Single-line Text (v4.2) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• True • False
Read 1 Cycles	Numeric (v4.2) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 151 • 101 • 51 • Range From: 1 to 151 • Decimal Places Displayed: 0
Read 2 Cycles	Numeric (v4.2) Numeric (v5)	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0
Run Mode *(not displayed in user interface)*	Single-line Text (v4.2) Text Dropdown (v5)	• Required: No • Read Only: Yes • Custom Entries: No	• S1 • S2 • S4
Sample Sheet Path	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: Yes	

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Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
Use Custom Index Read 1 Primer *LIMS v5.0.5 and later: See Configuration Updatedocumentation.*	Check Box (v4.2) Toggle Switch (v5)	• Required: No • Read Only: No	• None Set
Use Custom Read 1 Primer *LIMS v5.0.5 and later: See Configuration Updatedocumentation.*	Check Box (v4.2) Toggle Switch (v5)	• Required: No • Read Only: No	• None Set
Use Custom Read 2 Primer *LIMS v5.0.5 and later: See Configuration Updatedocumentation.*	Check Box (v4.2) Toggle Switch (v5)	• Required: No • Read Only: No	• None Set
Workflow	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: Yes	GenerateFASTQ
Workflow Type	Single-line Text (v4.2) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• No Index • Single Index • Dual Index • Custom

Global UDFs/custom fields

Table 15 lists the global UDFs / custom fields that are configured to display on the Load to Flowcell (NovaSeq 6000 v2.0) step.

Table 15: Global UDF / custom field configuration

Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
Configured on Analyte in LIMS v4.2 and Derived Samplein LIMS v5:			
Flowcell Type	• Single-line Text (v4.2) • Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• S1 • S2 • S4

User interaction

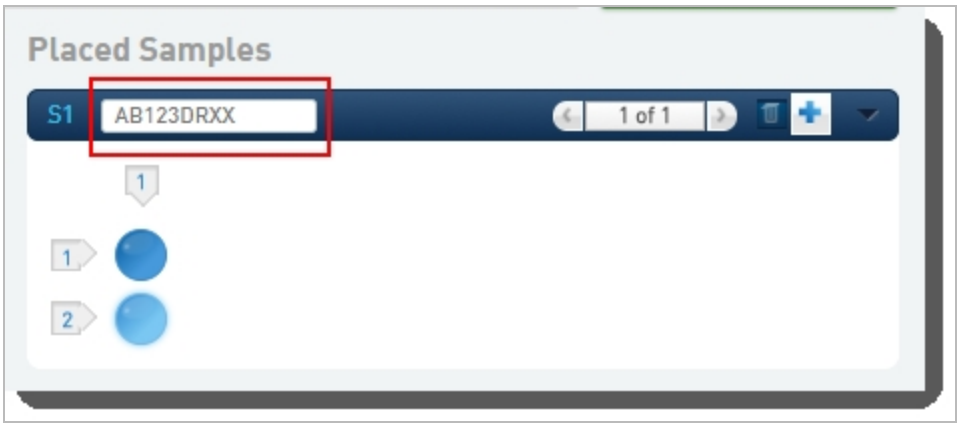
1. On the Ice Bucket screen, user: Selects the appropriate flow cell type from the Destination Container drop-down list in the Container Optionspanel.

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2. 

   - Clicks Begin Work.

3. On the Placement screen, user: Drags the pool(s) from the left of the screen over into the Placed Samples area on the right.
   - Scans or types the barcode of the flow cell into the flow cell field.



   - Clicks Record Details.On exit of the Placement screen, the Validate Flowcell Barcode automation validates the container barcode (described above).

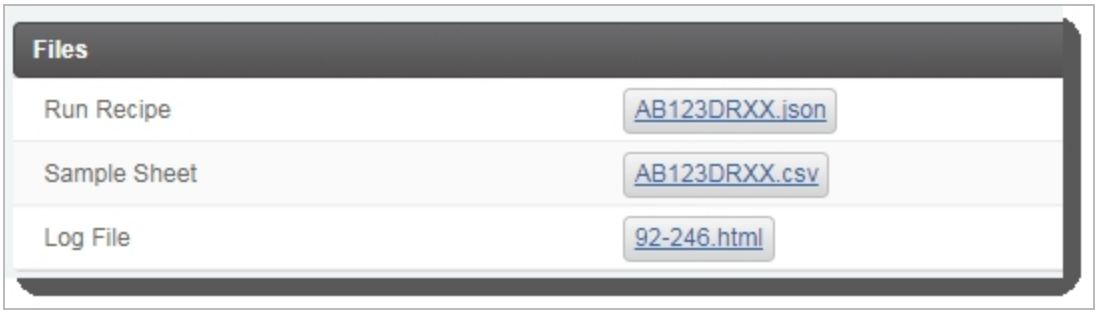
4. The fields displayed on the Record Details screen are used to generate the sample sheet and run recipe files. Some of these fields are auto-populated and some must be completed by the user (see Table 16, below, for details). *Table 16: Fields displayed on Record Details screen of Load to Flowcell (NovaSeq 6000 v2.0) step*

Field	Value
Experiment Name	User enters the name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.
BaseSpace Sequence Hub Configuration	User selects from preset options: Not Used, Run Monitoring Only, or Run Monitoring and Storage
Workflow	Automatically populated with preset - GenerateFASTQ.

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Field	Value
Workflow Type	User selects from preset options: No Index, Single Index, Dual Index, or Custom
Index Read 1	User selects from preset option: 0 , 6 , or 8 - or types a value between 0 and 20
Index Read 2	If user selected the Dual Index workflow type, once the sample sheet has been generated, Index Read 2 value is automatically populated to match Index Read 1.
Paired End	User selects from preset options: True or False
Read 1 Cycles	User selects from preset options: 151, 101, or 51 - or types a value between 1 and 151
Read 2 Cycles	If user selected Paired End =True, once the sample sheet has been generated, Read 2 Cycles value is automatically populated to match Read 1 Cycles.
Use Custom Read 1 Primer	User selects if applicable.
Use Custom Read 2 Primer	User selects if applicable.
Use Custom Index Read 1 Primer	User selects if applicable.
Sample Sheet Path	Automatically populated once the sample sheet has been generated.
Library Tube Barcode	User scans the library tube barcode.

5. On the Record Details screen, user clicks Generate Sample Sheet and Run Recipe . This triggers the automation script, which does the following:Generates the sample sheet and JSON files and attaches them to the placeholders in the Files area of the Record Detailsscreen.



   - Populates the Sample Sheet Path field with the path to the sample sheet file.
   - If applicable, populates the Index Read 2 and Read 2 Cycles fields.

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- Sets the value of the next step to Remove from workflow. The Routing Script automation expects this value, and requires it in order to successfully advance samples to the next step.

6. User clicks Next Steps. This triggers the Prepare Files for NovaSeq automation, which copies the sample sheet and run recipe files to the location specified during installation.
7. On the Assign Next Steps screen, the Next Step for all samples is pre-populated with Mark protocol as complete. User clicks Finish Step.
8. In Lab View, the pool of samples is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) step.

At this point in the workflow, the user interaction ends. The integration automatically starts the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) step and data from the run is parsed back into the LIMS.

Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0)

This final protocol contains one fully-automated step, AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0), described below.

 This is a fully-automated step. The user must not add samples to the Ice Bucket or start the step manually.

Step 1: AUTOMATED - NovaSeq Run (NovaSeq 6000) 1.0

Step input: Library tube from NovaSeq Standard protocol or NovaSeq Xp protocol

Step output: Result file / measurement

 The user must not start this step. This is a fully-automated step and the sequencing service will not update samples correctly if they have been manually started.

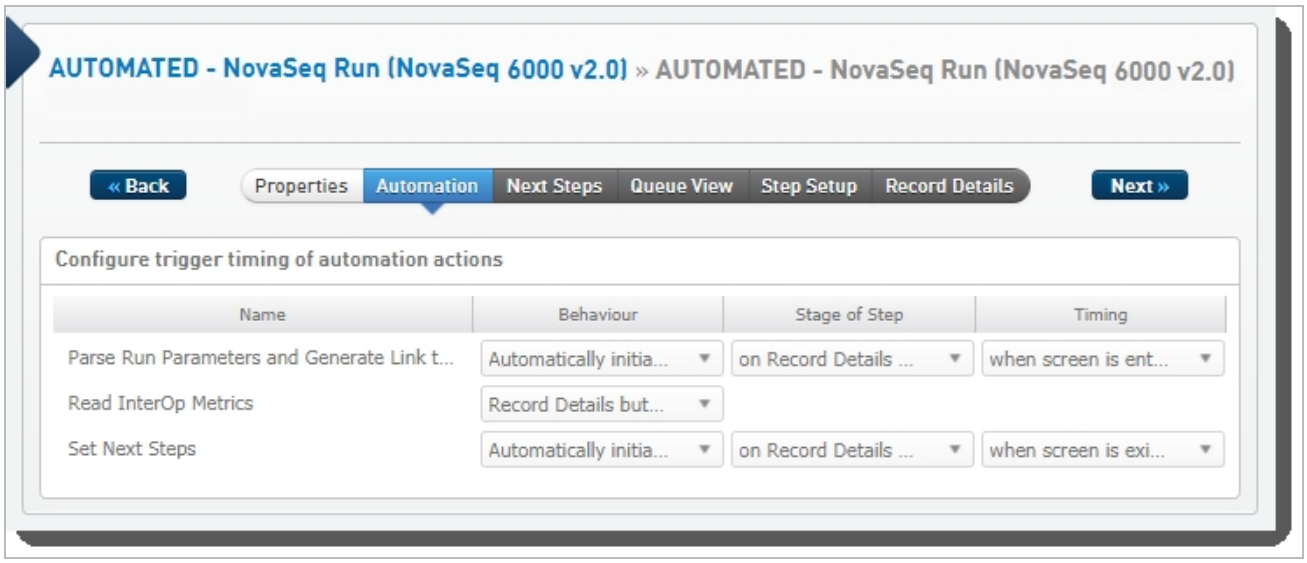
In this step, pooled samples are sequenced on the NovaSeq instrument. The run data network folder is monitored by the sequencing service and automation worker to determine when the sequencing run begins and ends.

1. When the user initiates a run on the NovaSeq instrument, the NovaSeq Control Software (NVCS) looks for the sample sheet and run recipe (*.json file) on the shared network drive.The NVCS copies the sample sheet to the run directory.
    - The NVCS uses the run recipe to start the sequencing run.
2. When the run starts: The instrument software creates a new run folder (named < libraryID >) on the shared network drive and copies the following files into that folder:RunParameters.xml
    - RunInfo.xml
    - LIMS/ <libraryID>.json file (run recipe)
        - The Real-Time Analysis v3 (RTA3) software creates the InterOp folder.
3. The sequencing service detects the presence of the RunParameters, RunInfo, and JSON files and starts the AUTOMATED- NovaSeq Run (NovaSeq 6000 v2.0) step in the LIMS.

4. As the run progresses, the InterOp data files are filled in.
5. At the end of the run, the NovaSeq: Copies the run data files to the InterOp folder.
    - Creates the CopyComplete.txt file in the run folder. This indicates to the sequencing service that the run has completed and the data files are ready.
6. The sequencing service triggers the Read InterOp Metrics automation, which reads the files, records the parsed metrics into the LIMS, and finally completes the step in LIMS.

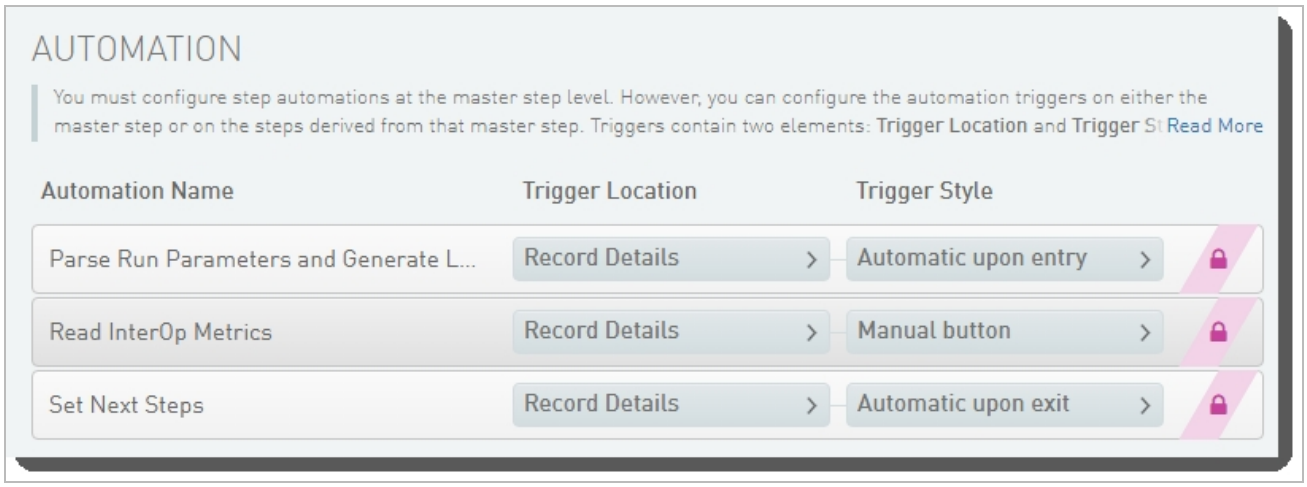
The following automations are configured on the step:

- Parse Run Parameters and Generate Link to Run Folder
- Read InterOp Metrics
- Set Next Steps



AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) automation configuration - LIMS v4.2

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AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) automation configuration - LIMS v5

These automations are described below, in the order in which they are triggered at run time.

Parse Run Parameters and Generate Link to Run Folder

Automatically triggered on entry to the Record Details screen, this automation:

- Parses the RunParameters.xml file attached on the Step Setup screen.
- Populates the step UDFs / custom fields with extracted values.
- If there is an instrument in the LIMS that matches the instrument name in the file (InstrumentName field), the script associates this instrument with the run.
- Generates and attaches a link to the run folder using the run output folder parsed from RunParameters.xml.

Rules and constraints

- If there is no InstrumentName value in the file, the script continues, but logs a warning.
- If there is an InstrumentName value in the file and multiple instruments with the same name exist in the LIMS, the script fails and logs an error.
- If the RunParameters.xml file is not attached to the step during step setup, the script fails.
- Every RunParameters.xml field that has an equivalent step UDF / custom field in the LIMS is required - except for InstrumentName. If a field is not present in the RunParameters.xml file, the LIMS logs an error to this effect.

Step UDFs/custom fields

Table 17, below, shows how the parsed RunParameters.xml fields map to step UDFs / custom fields configured on the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) step in the LIMS.

Table 17: Fields parsed by parse_novaseq_run_parameters script

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RunParameters.xml Data	*LIMS Step UDF / Custom Field Configuration*
RunParameters field	Step UDF / Custom Field Name
FlowCellSerialBarcode	Flow Cell ID
FlowCellPartNumber	Flow Cell Part Number
FlowCellLotNumber	Flow Cell Lot Number
FlowCellExpirationdate	Flow Cell Expiration Date
FlowCellMode	Flow Cell Mode
RunId	Run ID
ClusterSerialBarcode	PE Serial Barcode
ClusterPartNumber	PE Part Number
ClusterLotNumber	PE Lot Number
ClusterExpirationdate	PE Expiration Date
ClusterCycleKit	PE Cycle Kit
SbsSerialBarcode	SBS Serial Barcode
SbsPartNumber	SBS Part Number
SbsLotNumber	SBS Lot Number
SbsExpirationdate	SBS Expiration Date
SbsCycleKit	SBS Cycle Kit
BufferSerialBarcode	Buffer Serial Barcode
BufferPartNumber	Buffer Part Number
BufferLotNumber	Buffer Lot Number
BufferExpirationdate	Buffer Expiration
OutputRunFolder	Output Folder
WorkflowType	Loading Workflow Type

Read InterOp Metrics

Automatically triggered when the sequencing service detects the CopyComplete.txt file has been created, this automation parses the InterOp data generated by the run.

- Read summary metrics are recorded on the library pool. These values are aggregated across all lanes. Some values - for example Yield PF (Gb) - are summed, while others are averaged.
- Per-lane metrics are recorded on the measurement outputs.

The read_interop_metrics script loads data from the InterOp files and records summary metrics into derived sample UDFs / custom fields in the LIMS. The script will fail if any field is not configured or cannot be written to.

Global UDFs/custom fields

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The global UDFs / custom fields that are configured to support the parsing of key metrics back into the LIMS are as follows:

- Yield PF (Gb) R1
- Yield PF (Gb) R2
- Reads PF (M) R1
- Reads PF (M) R2
- Cluster Density (K/mm^2) R1
- Cluster Density (K/mm^2) R2
- %PF R1
- %PF R2
- % Bases >=Q30 R1
- % Bases >=Q30 R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- % Aligned R1
- % Aligned R
- % Error Rate R1
- % Error Rate R2

Set Next Steps

Automatically triggered on exit of the Record Details screen, this automation sets the next step to ADVANCE and the samples complete the protocol.

```
nextStep = ::ADVANCE::
```

Components installed with this integration

The following sections describe the various components - files, database properties, reagent categories / label groups, reagent kits, and containers - that are installed by default as part of this integration. Information on installed workflows, protocols, steps, and automation points is provided in the Workflows, protocols and steps section, above.

The Illumina NovaSeq 6000 Integration v2.2.1 is distributed as two RPM packages:

- BaseSpaceLIMS-novaseq-extensions

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- BaseSpaceLIMS-novaseq-sequencing-service

The RPM packages install the components listed in the following table.

Note that the NovaSeq 6000 Integration requires installation of NGS Extensions Package v5.15 or later.

- If the NGS Extensions Package is not already installed, or if a version earlier than than v5.15 is installed, v5.16 is installed by default with the NovaSeq integration. See Release Notes for details.
- If NGS Extensions Package v5.15 is already installed, upgrade is not forced or required.

Table 18: Illumina NovaSeq 6000 Integration RPM components

Files Installed	Location and Description
configure_extensions_novaseq.sh	*/opt/gls/clarity/config/* Script that imports LIMS configuration into the application database and updates property values.
novaseq-extensions.jar	*/opt/gls/clarity/extensions/novaseq* Jar file containing API-based LIMS extensions used throughout the protocols.
novaseq-sequencing.jar	*/opt/gls/clarity/extensions/novaseq/SequencingService* Sequencing service jar file that captures the run results.
novaseq-remote-extensions.jar	*/opt/gls/clarity/extensions/novaseq* Jar file containing scripts that require access to the run folders on the NAS.
InterOp libraries	*/opt/gls/clarity/extensions/novaseq/lib* Illumina shared library for parsing InterOp data files.
• NovaSeq_Standard_Bulk_Pool1.csv • NovaSeq_Standard_Bulk_Pool2.csv • NovaSeq_Standard_Bulk_Pool3.csv • NovaSeq_Xp_Bulk_Pool.csv • NovaSeq_Xp_Bulk_Pool2.csv • NovaSeq_Xp_Working_Pool.csv • NovaSeq_Xp_Working_Pool2.csv	*/opt/gls/clarity/extensions/novaseq/Templates* Template files used for file generation throughout the protocols.

Database properties

Table 19 lists the database properties installed with the Illumina NovaSeq 6000 Integration Package.

Table 19: Database properties installed

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Property	Description
novaseq.runSetupFolder	The directory where the run recipe files will be written.
novaseq.sampleSheetPathPrefixSearch.SUFFIX	The directory where the instrument control software or analysis software looks for sample sheets, typically a Windows path to a network drive.
novaseq.sampleSheetPathPrefixReplace.SUFFIX	The directory where the sample sheets will be written.
novaseq.sampleSheetPathPrefixSearchReplaceSuffixes*	Search and replace entries for transforming Windows to Linux network paths. This property must be set to the single SUFFIX used in the two properties above.
novaseq.seqservice.netPathPrefixSearch.SUFFIX	The search prefix used to map the OutputRunFolder field in RunParameters.xml to the corresponding Linux directory.
novaseq.seqservice.netPathPrefixReplace.SUFFIX	The directory where the sequencing service will look for run folders.
novaseq.seqservice.netPathPrefixSearchReplaceSuffixes*	Search and replace entries for transforming Windows to Linux network paths. This property must be set to the single SUFFIX used in the two properties above.

**This version of the integration only supports a single active search-replace pair of each type: sampleSheetPathPrefixSearchReplaceSuffixes and netPathPrefixSearchReplaceSuffixesmust each contain a single number, rather than a comma-separated list of numbers.*

Reagent categories/label groups

- TruSeq HT Adapters v2 (D7-D5)

Reagent kits

- DPX1
- DPX2
- DPX3
- Resuspension Buffer (RSB)
- NaOH

- Tris HCl

Container types

- Library Tube

Instrument integration

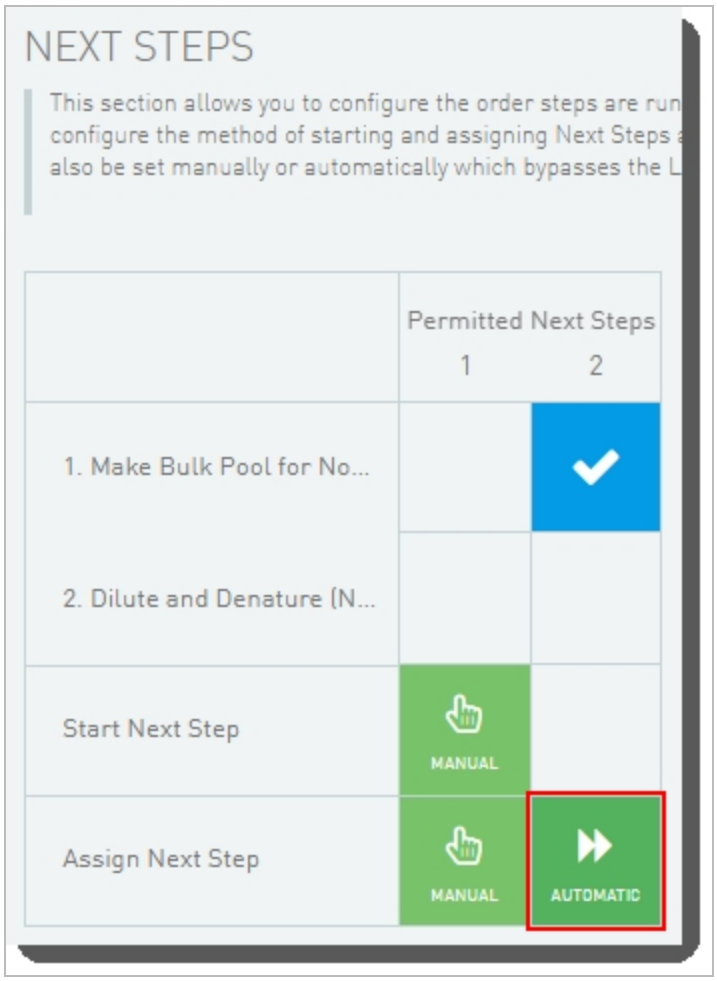
Instructions for configuring the NovaSeq instrument for LIMS integration are provided in the *NovaSeq Sequencing System Guide*.

Routing script requirements

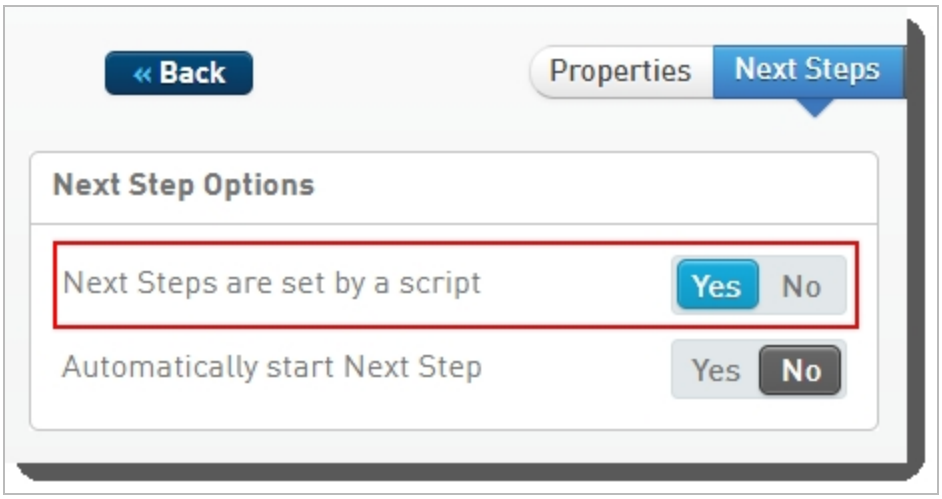
The requirements for the routing script functionality are as follows:

- On the steps that use the routing script - Define Run Format (NovaSeq 6000 v2.0) and Dilute and Denature (NovaSeq 6000 v2.0) - the Next Step for all samples must be set to Remove from workflow. This value is set by a script, and the user running the step must not change this value in the Assign Next Steps screen.
- LIMS v5: In the protocol configuration screen, the following settings are required and must not be changed:In the Next Steps section, for the last step in the protocol, the method of assigning the next step must be set to Automatic.

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- LIMS v4.2: In the protocol configuration area, for all protocol steps on which the Routing Script automation is configured, the following settings are required and must not be changed:
  On the Next Steps configuration tab, the Next Steps are set by a script toggle switch must be set to Yes.



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Sample sheet and run recipe file generation

Sample sheet and run recipe file generation occurs on the step prior to the run – Dilute and Denature (NovaSeq 6000 v2.0) in the NovaSeq Standard protocol or Load to Flowcell (NovaSeq 6000 v2.0) in the NovaSeq Xp protocol. This is the step where samples are placed on the library tube or flow cell that will be loaded in the NovaSeq instrument.

In the out-of-the-box configuration, the Generate Sample Sheet and Run Recipe automation generates the following:

- One CSV format sample sheet file for use bcl2fastq v2.19 downstream analysis
- One JSON format run recipe file

The Prepare Files for NovaSeq automation places the generated files on the NAS where the instrument will use them to start the run.

How it works

- The Prepare Files for NovaSeq automation command line invokes the copy_attachments_to_network_folder script in novaseq-remote-extensions.jar .The script may be used on a remote AI node / automation worker, in case the LIMS server does not have direct access to the NAS.
- The output folder for the run recipe file is found in the novaseq.runSetupFolder property.
- The output folder for the sample sheet is determined by using the novaseq.sampleSheetPathSearchReplaceSuffixes property. This property must contain exactly one suffix (not a comma-separated list) to derive a full property name based on novaseq.sampleSheetPathPrefixReplace.<suffix>.

Sample sheet contents

The contents of the sample sheet are controlled by the following fields configured on the Dilute and Denature (NovaSeq 6000 v2.0) and Load to Flowcell (NovaSeq 6000 v2.0) steps:

- Experiment Name
- Read 1 Cycles
- Read 2 Cycles
- Workflow

See the *Bcl2fastq2 Sample Sheet Generation* article for detailed information on the following:

- Sample sheet generation script parameters and usage
- Sample sheet data and configuration options
- Enabling unique FASTQ file names per sequencing run

Run recipe contents

The contents of the run recipe are controlled by the following fields. These fields are configured on the Dilute and Denature (NovaSeq 6000 v2.0) and Load to Flowcell (NovaSeq 6000 v2.0) steps.

- Experiment Name

- Run Mode
- Workflow Type
- Sample Loading Type (populated based on Workflow field)
- Paired End
- Read 1
- Read 2
- Index Read 1
- Index Read 2
- Sample Sheet Path
- BaseSpace Configuration
- Use Custom Read 1 Primer
- Use Custom Read 2 Primer
- Use Custom Index Read 1 Primer

For Dilute and Denature (NovaSeq 6000 v2.0) step field configuration details, see Table 7. To see how these fields are populated on the Record Details screen at run time, see Table 9.

For Load to Flowcell (NovaSeq 6000 v2.0) step field configuration details, see Table 14. To see how these fields are populated on the Record Details screen at run time, see Table 16.

The following fields are also included in the run recipe:

- Output Container Name (*librarytube_ID* in JSON file): The library tube barcode derived from the container name entered or scanned on the Placement screen
- Rehyb - preset to False
- Output Folde r (*output_folder* in JSON file): Derived from the system property novaseq.seqservice.netPathPrefixSearch
- Attachment: Derived from the system property novaseq.sampleSheetPathPrefixSearch with the name of the sample sheet added to the end of the path
- Use BaseSpace (*use_basespace* in JSON file): Set to False if the BaseSpace Sequence Hub Configuration step UDF / custom field value is 'Not Used', and true otherwise

Rules and constraints

- The sample sheet and run recipe files are mandatory inputs for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) step. For the automated run to start successfully, the user must have clicked the Generate Sample Sheet and Run Recipe button to generate these files.
- The output folders must exist on the system where the script runs (this may be a remote AI node / automation worker).
- The output folders must be writable by the glsai user.

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Support for container types

This integration supports the following container types:

- Library tube, with barcode provided in the format [A-Za-z]{2}[0-9]{7}-[A-Za-z]{3}Example: AB1234567-XYZ
- Flow cell , with barcode provided in one of the following formats:[A-Za-z0-9]{5}DRXX
  - [A-Za-z0-9]{5}DMXX
  - [A-Za-z0-9]{5}DSXX

Example: AB123DRXX

Rules and constraints

- The workflow configuration contains several validation checks. To ensure that the calculations work properly, it is important that you do not disable any of this validation logic. The validation checks determine:Which samples, and how many, can enter each step together.
  - Which samples, and how many, can be pooled together.
- Reagent labels (indexes) must be unique.
- The library tube ID must be unique. There should not be multiple library tube containers in the system with the same name.
- Only controls are permitted as unindexed samples; all other unindexed samples and pools are not permitted.
- For sample sheet generation constraints, refer to the *Bcl2fastq2 Sample Sheet Generation* article.
- Users must not manually start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) step. This is a fully-automated step and the sequencing service will not update samples correctly if they have been manually started.
- The output folder must be readable by the LIMS server's glsjboss account. For details, see *Installation Guide*.

NovaSeq 6000 Integration v2.2.1 Validation and Troubleshooting

This guide explains how to validate the installation of the Illumina NovaSeq 6000 Integration Package v2.2.1.

The validation process involves:

- Running samples through the NovaSeq Validation Library Prep (NovaSeq 6000 v2.0) workflow. The workflow contains a single-step protocol that models the library prep required to produce normalized libraries. At the end of the step, the normalized libraries are automatically advanced to the NovaSeq 6000 v2.0 workflow.

- Running normalized libraries through the NovaSeq 6000 v2.0 workflow. This validates the following: Successful routing of samples from the Run Format (NovaSeq 6000 v2.0) step to the NovaSeq Standard (NovaSeq 6000 v2.0) or NovaSeq Xp (NovaSeq 6000 v2.0) step.
  - Automated generation of a sample sheet for use with bcl2fastq2 v2.19 analysis software.
  - Automated generation of a run recipe file (JSON file) with the library tube or flow cell barcode as the name (e.g., AB1234567-CDE.json). This file is automatically uploaded to the sequencing instrument and used to set up and initiate the run.
  - Automated tracking of the NovaSeq sequencing run and parsing of run statistics (per run per lane) into the LIMS.

Before you begin

Before you begin to execute the validation steps below, note that these steps assume that you have installed the Illumina NovaSeq 6000 Integration Package v2.2.1 and have imported the default LIMS configuration.

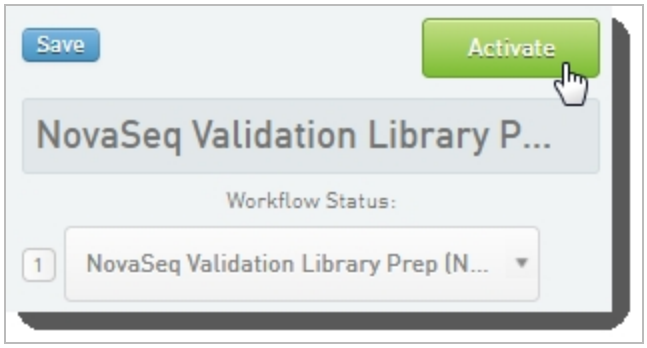
Validation steps

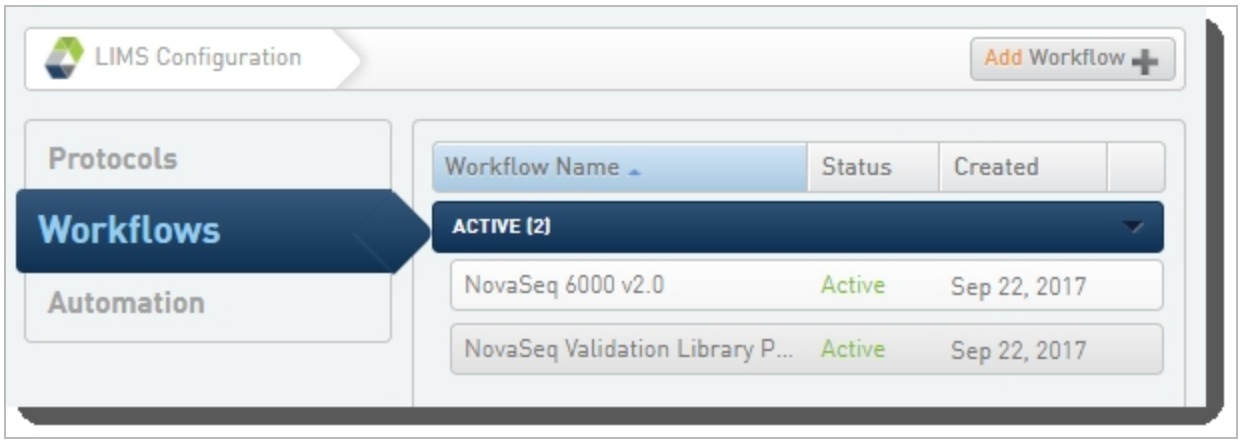
Activate workflow, create project, add and assign samples

The following steps set up the LIMS in preparation for running samples through the NovaSeq Validation Library Prep (NovaSeq 6000 v2.0) and NovaSeq 6000 v2.0 workflows.

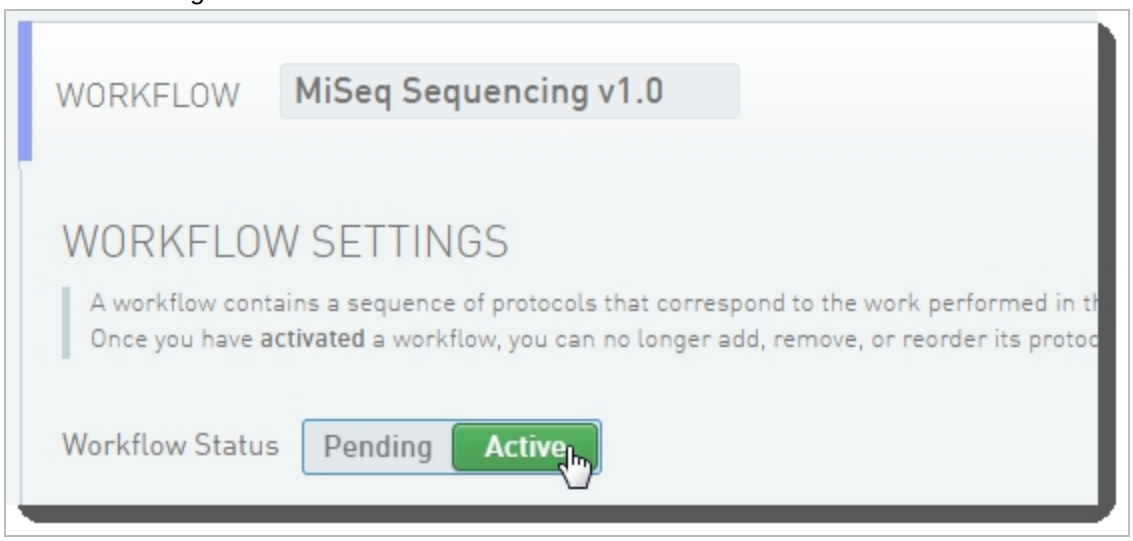
1. Activate the workflow

- In the LIMS configuration area, activate the NovaSeq Validation Library Prep (NovaSeq 6000 v2.0) and NovaSeq 6000 v2.0 workflows.





Workflow configuration and activation - LIMS v4.2

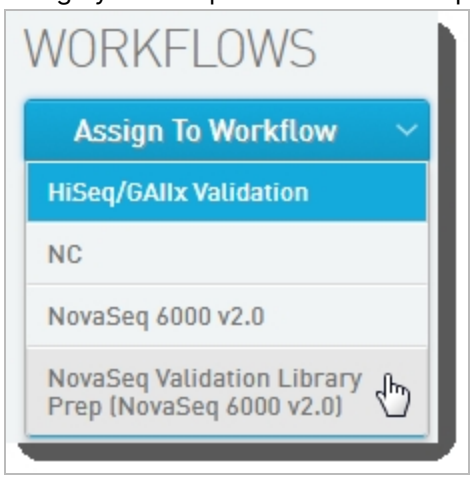


Workflow configuration and activation - LIMS v5.0

2. Create a project, add samples

1. On the Projects and Samples screen, create a project and add samples to it.

2. Assign your samples to the NovaSeq Validation Library Prep (NovaSeq 6000 v2.0)workflow.

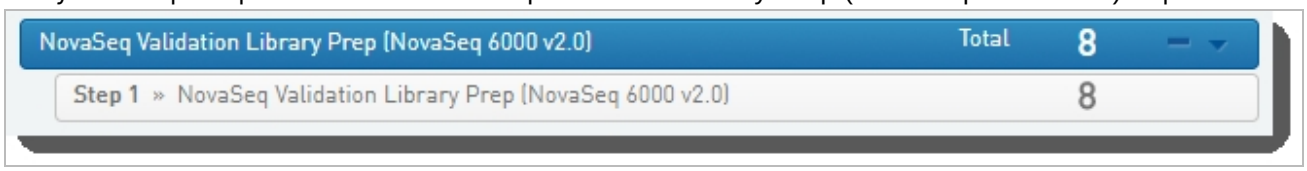


Library Prep Protocol: NovaSeq Validation Library Prep (NovaSeq 6000 v2.0)

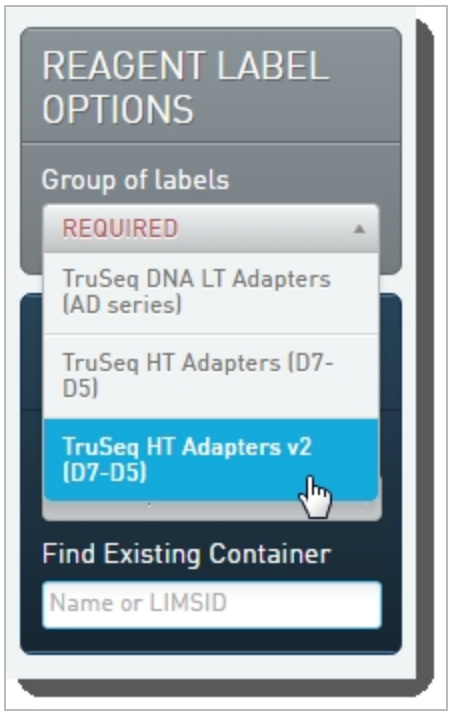
This single-step protocol models the library prep required to produce normalized libraries that are ready for the NovaSeq 6000 v2.0 workflow.

Run NovaSeq Validation Library Prep (NovaSeq 6000 v2.0) step

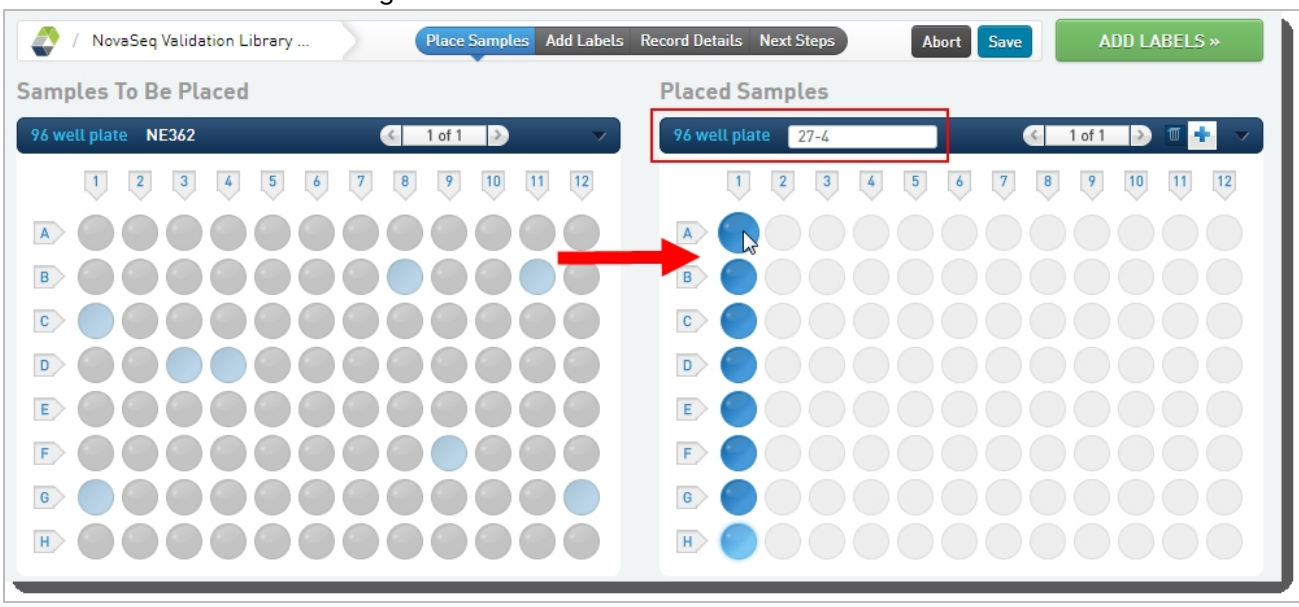
1. In Lab View, locate the NovaSeq Validation Library Prep (NovaSeq 6000 v2.0) protocol. You will see your samples queued for the NovaSeq Validation Library Prep (NovaSeq 6000 v2.0)step.



2. Add the samples to the Ice Bucket and click View Ice Bucket.
3. On the Ice Bucket screen, in the Reagent Label Options panel, select a group of reagent labels from the drop-down list. Click Begin Work.

4. 

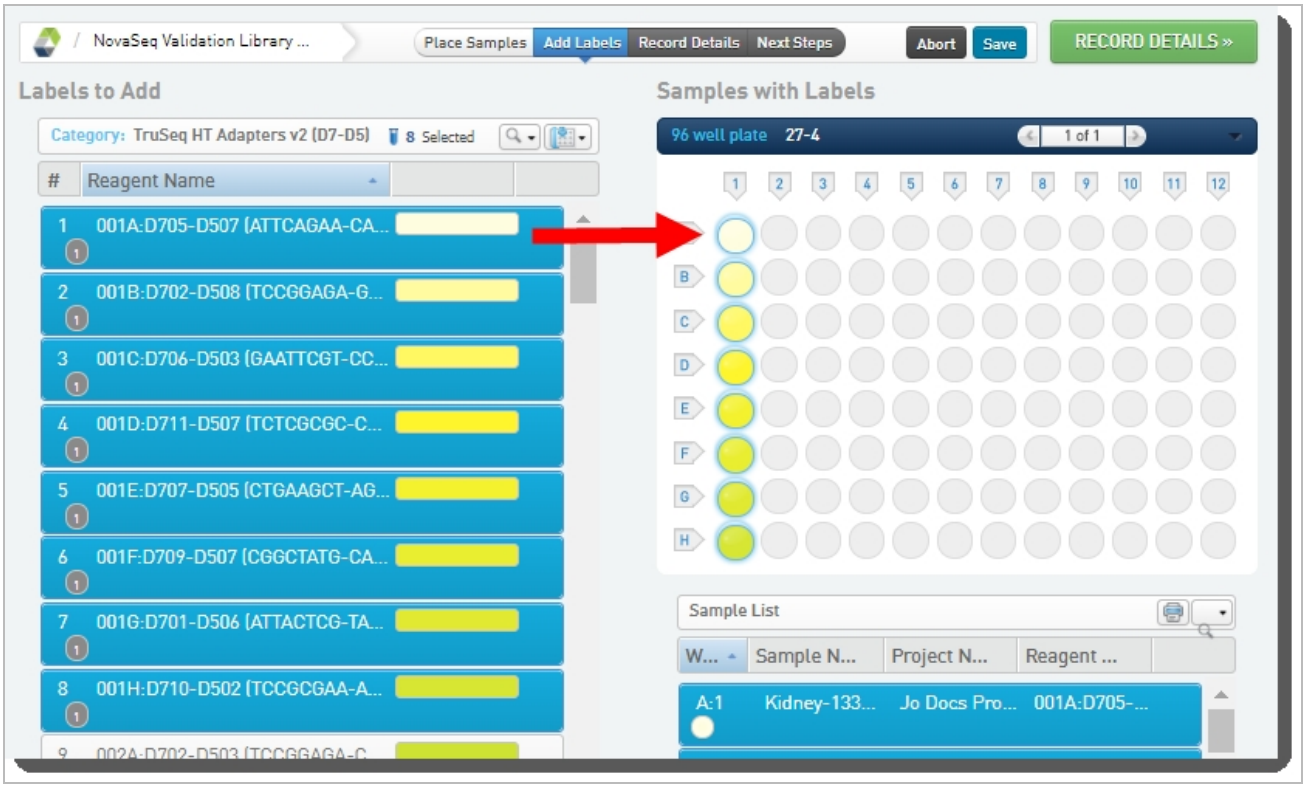
5. On the Placement screen: In the Placed Samples area on the right, in the container field, scan or enter the barcode of the destination container.
    - Select the samples from the Samples to be Placedarea on the left, and then drag them over to the container wells on the right.



    - Click Add Labels.

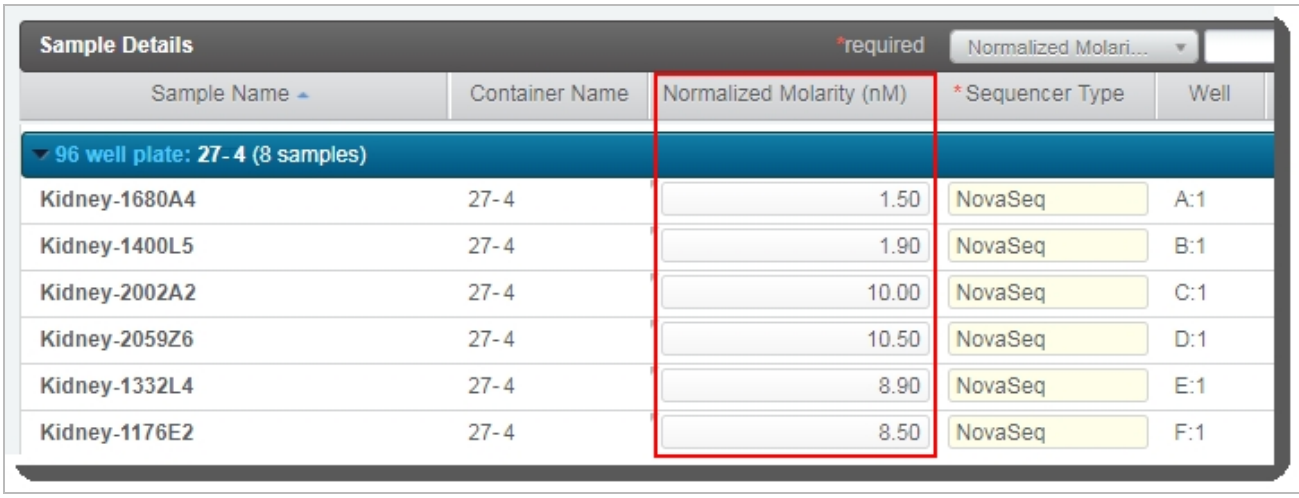
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6. On the Add Labels screen, the Labels to Add list is populated with labels from the TruSeq HT Adapters v2 (D7-D5) label group. This is the only label group configured in the out-of-the-box integration and it is used by default in this step.Select labels on the left, and drag them over to the container on the right to assign them to placed samples.



- Click Record Details.

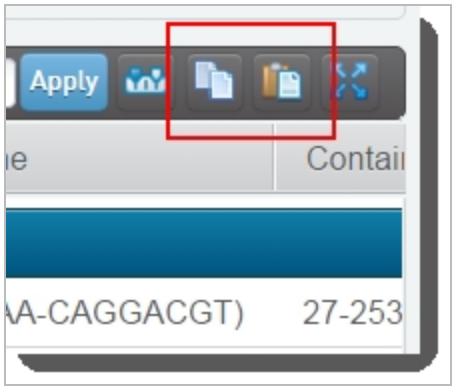
7. On the Record Details screen:
   - In the Sample Details table, enter values for Normalized Molarity. You can type values individually, or use Copy / Paste functionality to enter multiple values from a spreadsheet (see Tipbelow).



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- The Sequencer Type field indicates the instrument to be used for the sequencing run. In this integration, the value of this field is automatically set to NovaSeq.

Tip:
If you are working with a large number of samples, you can use Copy / Paste functionality to enter multiple values from a spreadsheet.
1. Click the Copy Samples button to copy the sample information from the LIMS to the Clipboard.
2. Open a blank Excel document and paste the contents of the Clipboard into the spreadsheet.



3. In the spreadsheet, enter the Normalized Molarity values. Select the entire spreadsheet, including the headers (Ctrl + A), and copy (Ctrl + C) the information to the Clipboard.
4. In the LIMS, click the Paste Samples button. Paste the contents of the clipboard (Ctrl + V) into the Update Table window and then click Update to paste into the S ample Details table.

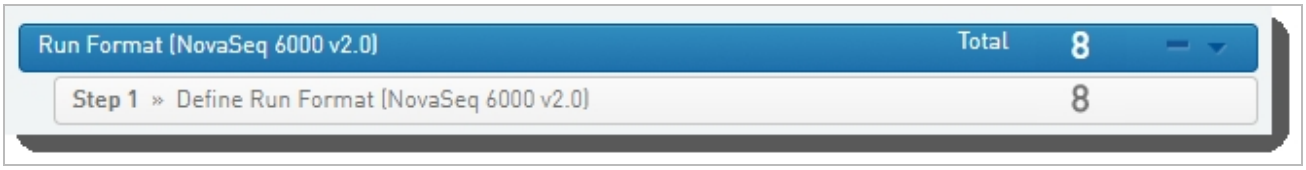
8. Click Next Steps.
   - This triggers the Next Steps automation, which sets the value of the next step (for all samples) to Remove from workflow. Note that the Routing Script automation expects this value and requires it in order to successfully advance samples to the next step.
9. On the Assign Next Steps screen, in the Samples table, the Next Step for all samples is pre-populated with Remove from workflow.
   Do not change this value! If Next Step is not set to Remove from workflow, the routing script will not be able to route samples correctly.
10. Click Finish Step.On exit from the step, the Routing Script automation is triggered. This automation assigns samples to the first step of the NovaSeq 6000 v2.0 workflow - Define Run Format (NovaSeq 6000 v2.0). This is the only step in Protocol 1: Run Format (NovaSeq 6000 v2.0).

Protocol 1: Run Format (NovaSeq 6000 v2.0)

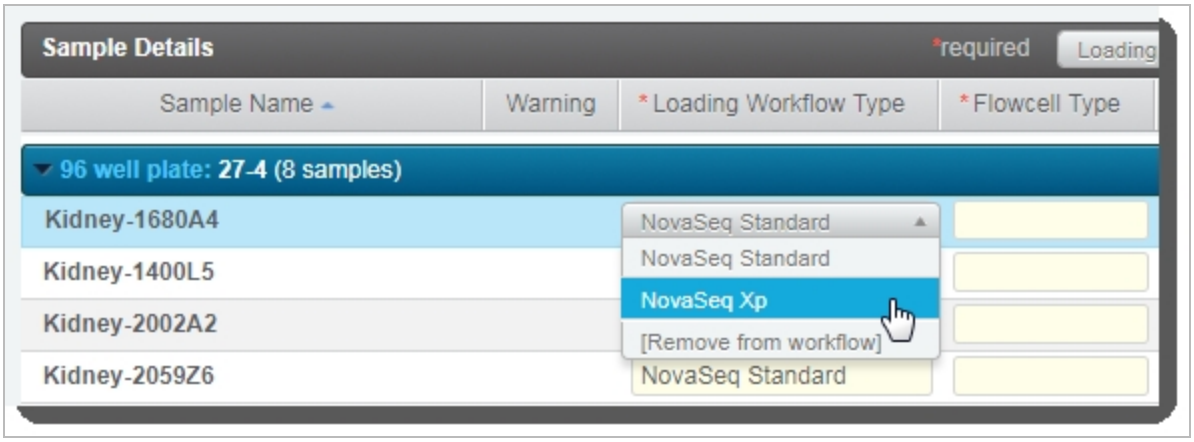
This protocol includes a single step - Define Run Format (NovaSeq 6000 v2.0). The step allows for the assignment of per sample values for Loading Workflow Type, Normalized Molarity, Flowcell Type, and Final Loading Concentration (pM). At the end of the step, samples are routed to the NovaSeq Standard or NovaSeq Xp protocol, according to the selected Loading Workflow Type.

Run Define Run Format (NovaSeq 6000 v2.0) step

1. In Lab View, locate the Run Format (NovaSeq 6000 v2.0) protocol. You will see your samples queued for the Define Run Format (NovaSeq 6000 v2.0)step.



2. Add the samples to the Ice Bucket and begin work.
3. On the Record Details screen, in the Sample Details table:Loading Workflow Type: Select NovaSeq Standard or NovaSeq Xpfrom the drop-down list.



   - Normalized Molarity: These values are copied over from the previous step. (If the user did not populate this column during library prep, they can enter the values here.)
4. Populate the following fields (values can vary across samples):Flowcell Type: Select S1, S2, or S4.
   - Final Loading Concentration (pM): You may select from the two preset options - 225 (for PCR-free workflows) or400 (for Nano workflows), or you may enter a different value.
5. Click Next Steps. This triggers the Next Steps automation, which does the following: Sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value, and requires it in order to successfully advance samples to the next step.
   - Calculates the Minimum Molarity.
   - Checks Normalized Molarity value. For samples with no Normalized Molarity value (i.e., empty value, not including 0), generates an error message informing the user that the field cannot be empty.
   - Compares each sample's Normalized Molarity value with the Minimum Molarity value.
6. On the Assign Next Steps screen: In the Sample Details table, the Next Step for all samples is pre-populated with Remove from workflow - regardless of the Loading Workflow Type. Do not change this value! If Next Step is not set to Remove from workflow, the routing script will not be able to route samples correctly.

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- For samples whose Normalized Molarity value was found to be lower than the Minimum Molarity value, the Loading Workflow Type is set to Remove from workflow and a message is recorded in the Warning field for the sample. At this point you have two options: Return to the Record Details screen and adjust the Normalized Molarity value so that it equals or exceeds the Minimum Molarity value. You will also need to set the Loading Workflow Type to NovaSeq Standard or NovaSeq Xp, as applicable.

– Complete the protocol without correcting the Normalized Molarity value. In this case, the samples in question will be removed from the LIMS workflow.

7. Click Finish Step. The Routing Script automation is triggered: Samples whose Loading Workflow Type is set to Remove from workflow (i.e., whereNormalized Molarity value is lower than the Minimum Molarity) are removed from the LIMS workflow.
   - Samples whose Loading Workflow Type is NovaSeq Standard are routed to the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0) step. This is the first of two steps in Protocol 2: NovaSeq Standard (NovaSeq 6000 v2.0).
   - Samples whose Loading Workflow Type is set to NovaSeq Xp are routed to the Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.0) step. This is the first of three steps in Protocol 3: NovaSeq Xp (NovaSeq 6000 v2.0).

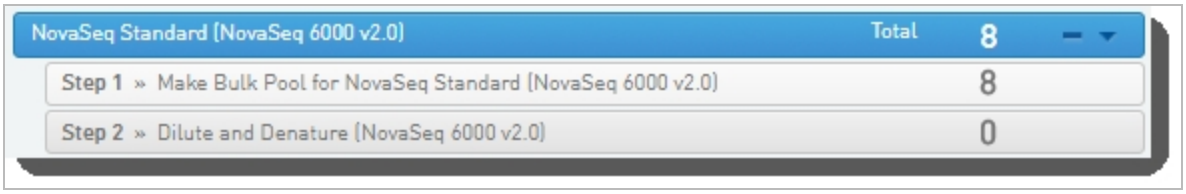
Protocol 2: NovaSeq Standard (NovaSeq 6000 v2.0)

In this protocol, samples are pooled and added to the library tube in preparation for the NovaSeq run. The protocol contains two steps:

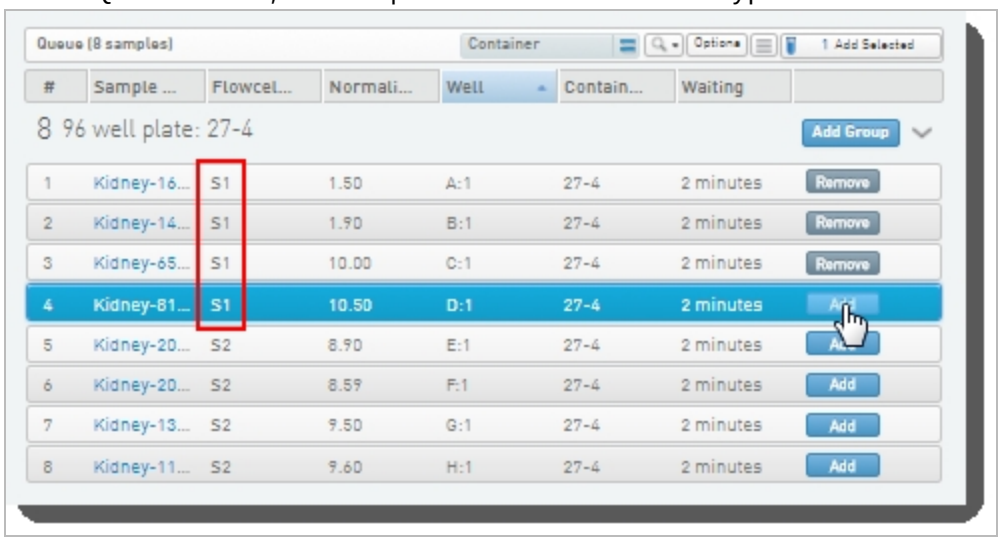
1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0)
2. Dilute and Denature (NovaSeq 6000 v2.0)

1. Run Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0) step

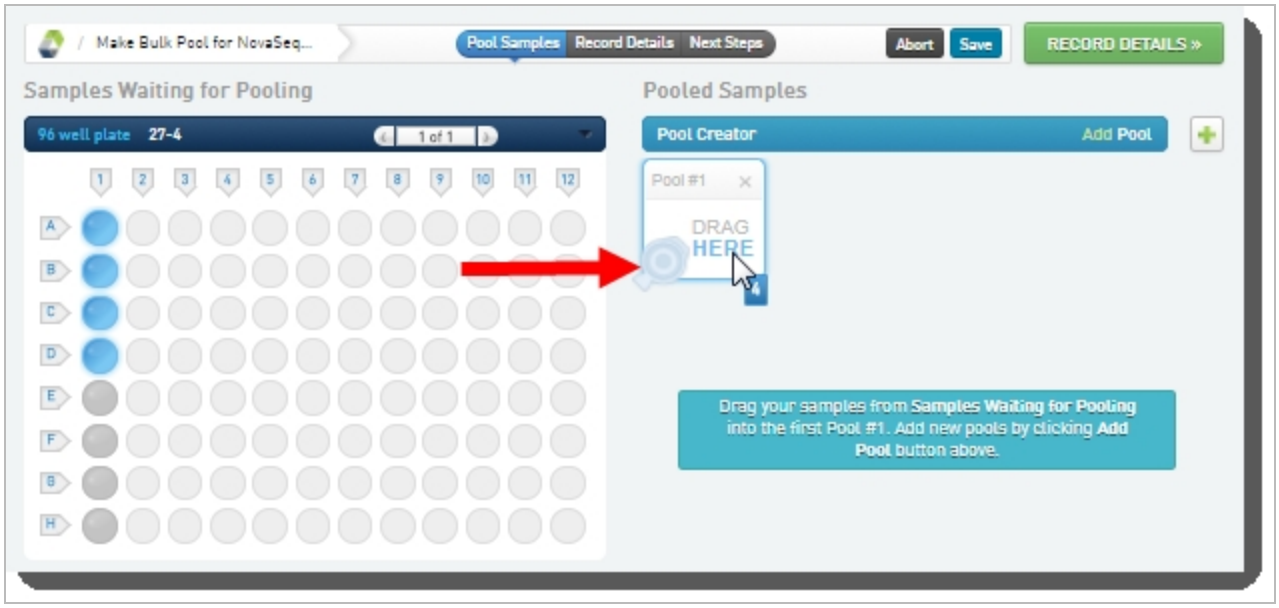
1. In Lab View, locate the NovaSeq Standard (NovaSeq 6000 v2.0) protocol. You will see your samples queued for the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0)step.



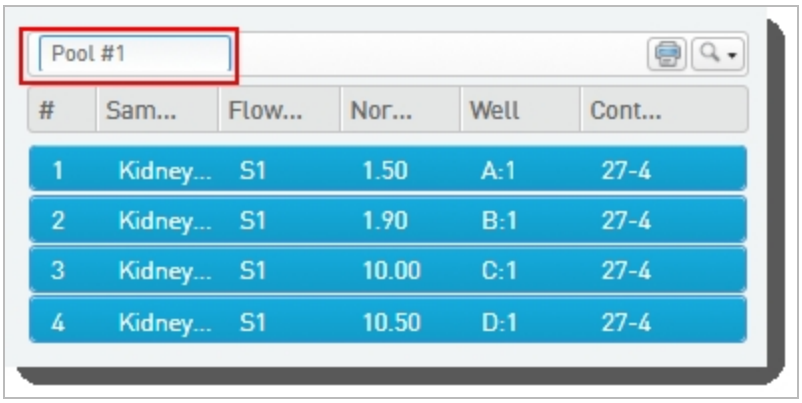
2. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucketand begin work.



3. On the Pooling screen: Create a pool by dragging samples into the Pool Creator. Note that only one pool must be created.



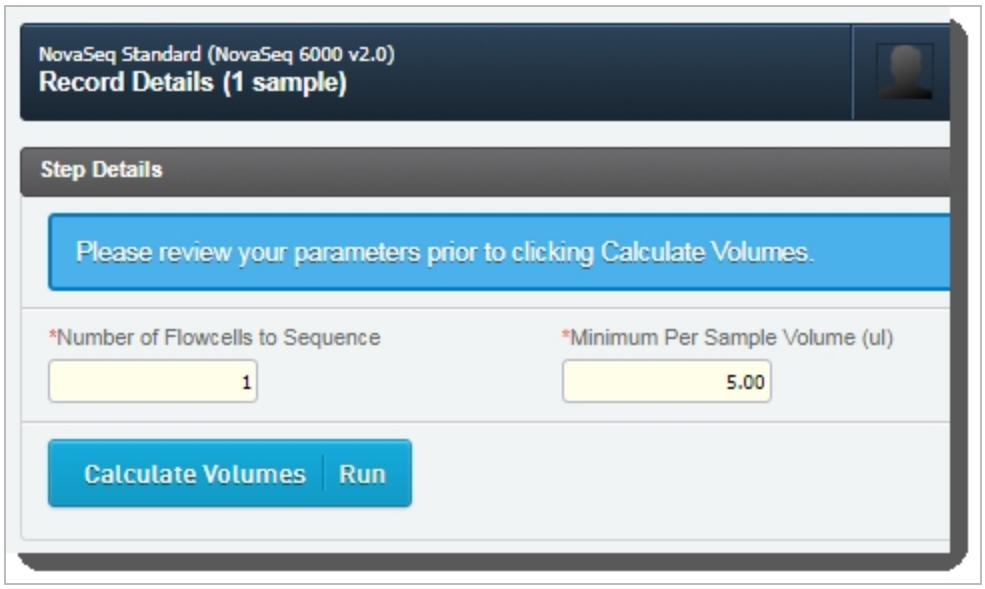
- You can type a name for the pool, or accept the default name - Pool #1.



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- Click Record Details.

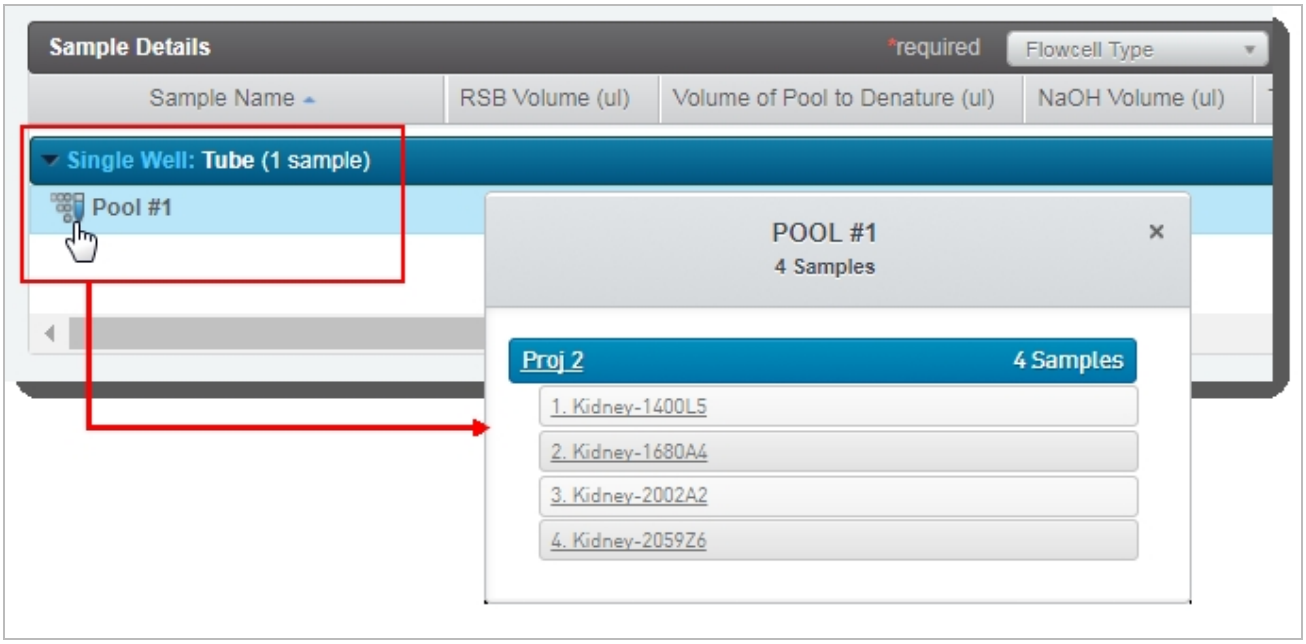
4. On exit from the Pooling screen, the Validate Inputs Flowcell Type and Single Pool is triggered. The automation checks that: All samples in the pool have been assigned the same Flowcell Type.
    - Only one pool has been created.
5. On the Record Details screen, the Step Details area contains two required fields:Number of Flowcells to Sequence: The value entered in this field is used in volume calculations, to ensure that volumes are sufficient for the number of times the pool will be sequenced.
    - Minimum Per Sample Volume (ul): The value in this field is used to calculate how much of each sample will be included in the pool. The field is pre-populated with the configured default value - 5 ul, but may be edited if required.



Note:
Assuming the default Minimum Per Sample Volume (ul) value of 5, for a given batch:
1. If the smallest Per Sample Volume (ul) value is less than 5, the LIMS automatically assigns a value of 5 to the sample's Adjusted Per Sample Volume (ul) field.
2. The LIMS then adjusts the Adjusted Per Sample Volume (ul) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

- In the Sample Details table, you can click on the pool icon to view details on the pool composition.

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- Click Calculate Volumes. This triggers the Calculate Volumes automation, which performs volume calculations based on the selected Flowcell Type : Calculates Bulk Pool Volume (ul) value.

– Calculates the Per Sample Volume (ul) value to be added to the pool.
– Calculates the Total Sample Volume (ul) value.
– If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (ul) value.
– Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
– Populates the Volume of Pool to Denature (ul), NaOH Volume (ul) and Tris-HCl Volume (ul) columns of the Sample Details table. (Values are set by a script and are not editable by the user.)
– The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains volume information about the pool and the individual samples it contains. Click on the file to download it and then open it in Excel.

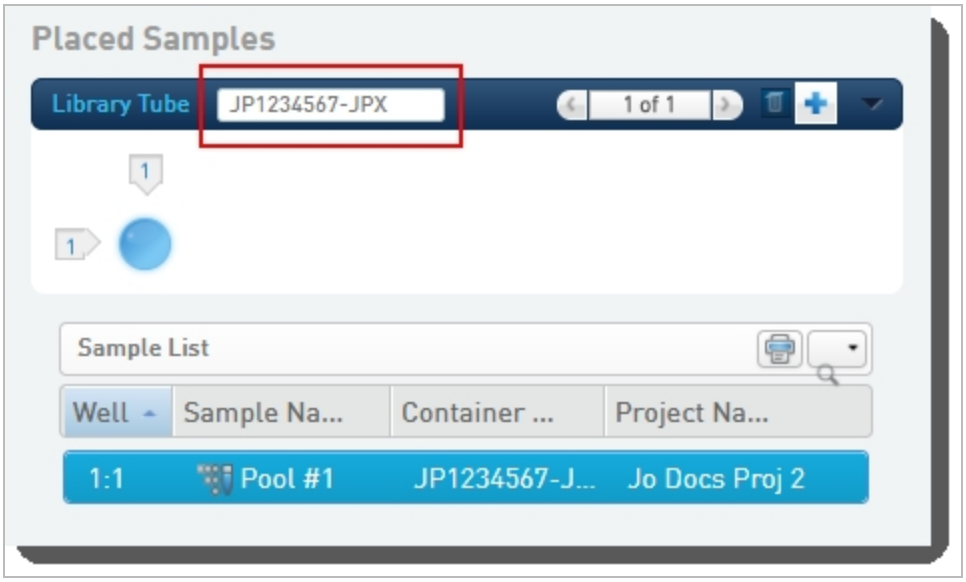
- Click Next Steps. This triggers the Set Next Step automation. This automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol - Dilute and Denature (NovaSeq 6000 v2.0).

6. On the Assign Next Steps screen, the next step for samples is already set to the next step in the workflow - Dilute and Denature (NovaSeq 6000 v2.0).
7. Click Finish Step.

At the end of this step, the pool of samples automatically advances to the Dilute and Denature (NovaSeq 6000 v2.0) step.

2. Run Dilute and Denature (NovaSeq 6000 v2.0) step

1. In Lab View, locate the NovaSeq Standard (NovaSeq 6000 v2.0) protocol. You will see the pool of samples queued for the Dilute and Denature (NovaSeq 6000 v2.0) step.
2. Add the samples to the Ice Bucket and begin work. At the beginning of the step, the Validate Single Input automation is triggered. This automation checks that there is only one container input to the step.
3. On the Placement screen: Drag the pool into the library tube in the Placed Samples area.
    - Scan or type the barcode of the library tube into the Library Tubefield.

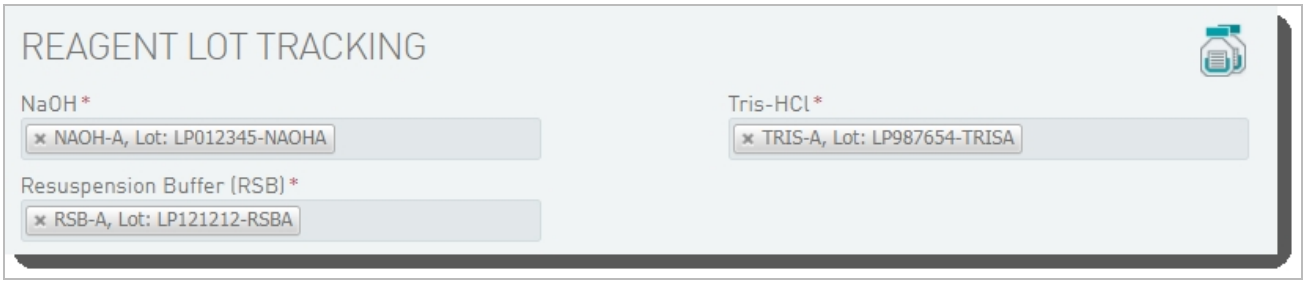


    - Click Record Details.On exit of the Placement screen, the Validate Library Tube Barcode automation checks that the library tube barcode conforms to the barcode mask [A-Za-z]{2}[0-9]{7}-[A-Za-z]{3}, and displays an error message if this is not the case.
4. On the Record Details screen, the Reagent Lot Tracking section lets you track the NaOH, Resuspension Buffer, and Tris-HCI reagents used in the step. You will need to add and activate lots for these reagents. To add and activate reagent lots:
    - In a new browser tab, open an additional instance of the LIMS. LIMS v5.0: Navigate to the Consumables configuration screen.

    – LIMS v4.2: Navigate to the Reagents and Controls screen.
        - Select the NaOH reagent kit and click New Lot.
        - Enter the lot details and click Save.
        - Set the Status of Reagent Lot toggle switch to Active.
        - Repeat this process to add and activate Resuspension Buffer and Tris-HCI reagent lots.
        - Close this browser tab and return to your original working tab. Refresh the page.

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5. In the Reagent Lot Tracking section, select from the active lots displayed in each drop-down list.



The fields displayed in the Step Details section are used to generate the sample sheet and run recipe files. Some of these fields are auto-populated and some must be completed by the user (see table below for details). *Fields displayed on Record Details screen of Denature and Dilute (NovaSeq 6000 v2.0) step*

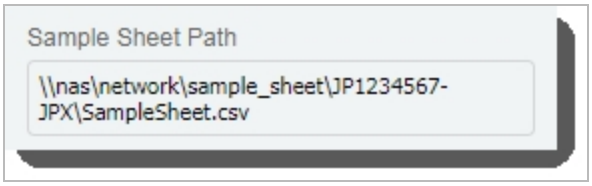
Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. No spaces.
BaseSpace Sequence Hub Configuration	Select from preset options: Not Used, Run Monitoring Only, or Run Monitoring and Storage
Workflow Type	Select from preset options: No Index, Single Index, Dual Index, or Custom
Index Read 1	Select from preset options: 0, 6, or 8 - or type a value between 0 and 20.
Index Read 2	If Dual Index workflow type selected, once the sample sheet has been generated, Index Read 2 value is automatically populated to match Index Read 1.
Paired End	Select from preset options: True or False
Read 1 Cycles	Select from preset options: 151, 101, or 51 - or type a value between 1 and 151.
Read 2 Cycles	If Paired End = True, once the sample sheet has been generated, Read 2 Cycles value is automatically populated to match Read 1 Cycles.
Use Custom Read 1 Primer	Select if applicable. LIMS v5.0.5 and later: See the *Configuration Update* documentation.
Use Custom Read 2 Primer	Select if applicable. LIMS v5.0.5 and later: See the *Configuration Update* documentation.
Use Custom Index Read 1 Primer	Select if applicable. LIMS v5.0.5 and later: See the *Configuration Update* documentation.
Sample Sheet Path	Automatically populated once the sample sheet has been generated.
Workflow	Automatically populated with preset - GenerateFASTQ.

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6. On the Record Details screen, click Generate Sample Sheet and Run Recipe . This triggers the automation script: The sample sheet and JSON files are generated and attached to the placeholders in the Files area of the Record Detailsscreen.



   - The Sample Sheet Pathfield is populated with the path to the sample sheet file.



   - If applicable, the Index Read 2 and Read 2 Cycles fields are populated.
   - Click Next Steps. This triggers the Prepare Files for NovaSeq automation which does the following:Copies the sample sheet and run recipe files to the location specified during installation. The NovaSeq instrument software uses these files to set up the run.

   – Sets the value of the next step to Remove from workflow. The Routing Script automation expects this value, and requires it in order to successfully advance samples to the next step.

7. On the Assign Next Steps screen: The Next Step for samples is pre-populated with Remove from workflow. ⚠Do not change this value! If Next Step is not set to Remove from workflow, the routing script will not be able to route samples correctly.
8. Click Finish Step .On exit from the step: The Routing Script automation is triggered and samples are routed to Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0).
   - In Lab View, the pool of samples is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) step.

At this point in the workflow, the user interaction ends. Proceed to Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0).

⚠Do not add samples to the Ice Bucket or start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) step. The integration will do this automatically.

Protocol 3: NovaSeq Xp (NovaSeq 6000 v2.0)

In this protocol, samples are pooled and added to lanes on the NovaSeq flow cell. The flow cell type is determined by the option selected in the Define Run Format (NovaSeq 6000 v2.0) step.

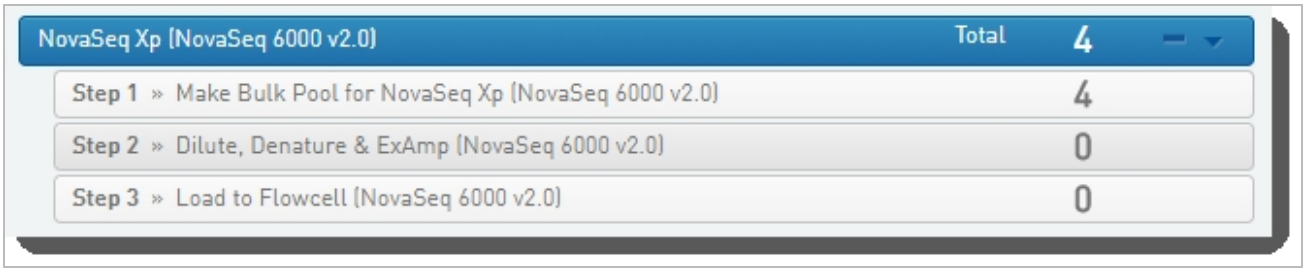
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The protocol contains three steps:

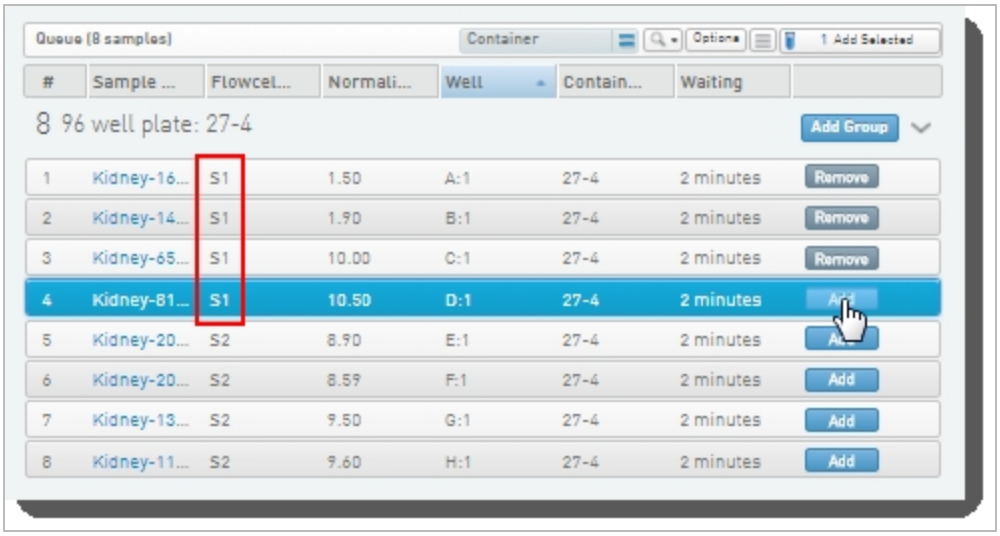
1. Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.0)
2. Dilute, Denature and ExAmp (NovaSeq 6000 v2.0)
3. Load to Flowcell (NovaSeq 6000 v2.0)

1. Run Make Bulk Pool for NovaSeq Xp (NovaSeq 6000 v2.0) step

1. In Lab View, locate the NovaSeq Standard (NovaSeq 6000 v2.0) protocol. You will see your samples queued for the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0)step.

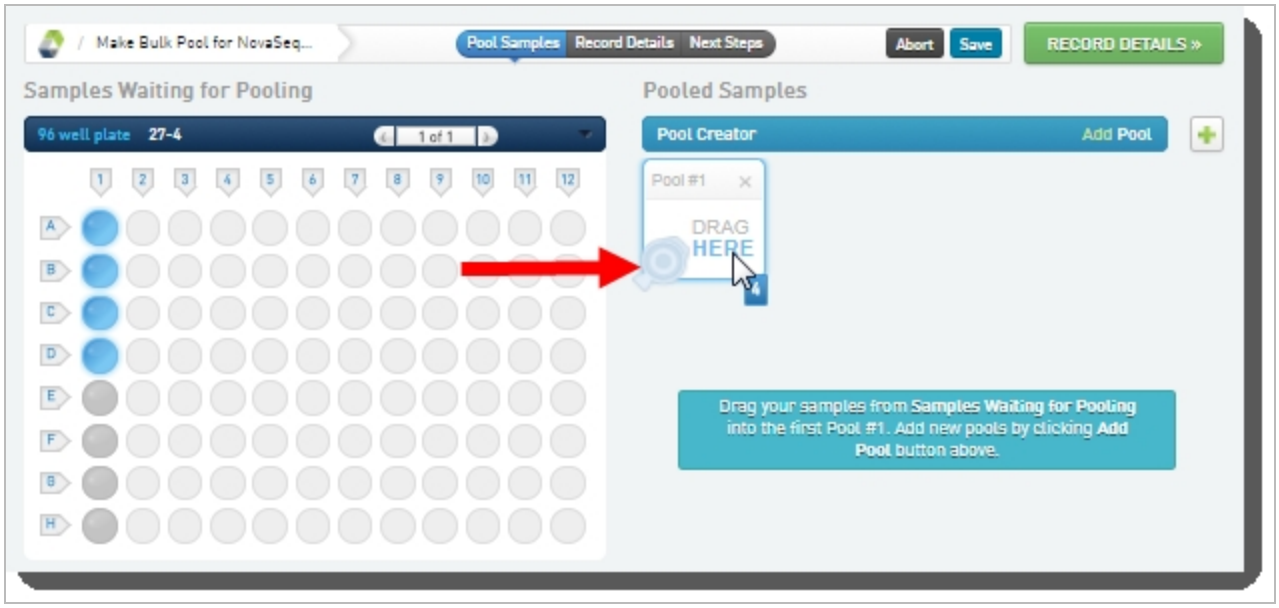


2. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucketand begin work.

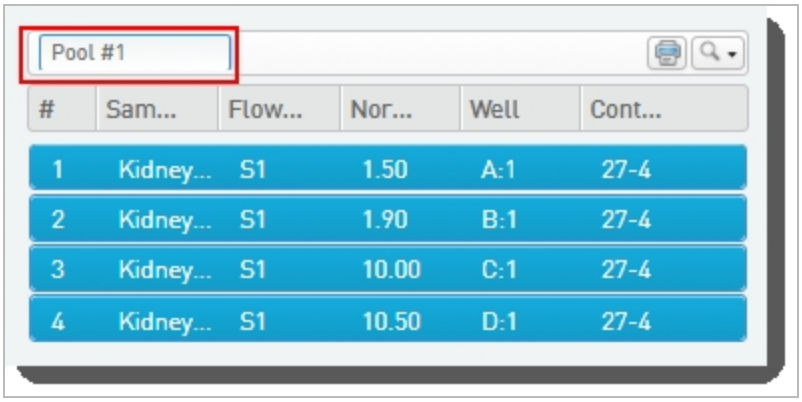


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3. On the Pooling screen: Create a pool by dragging samples into the Pool Creator. Note that only one pool must be created.



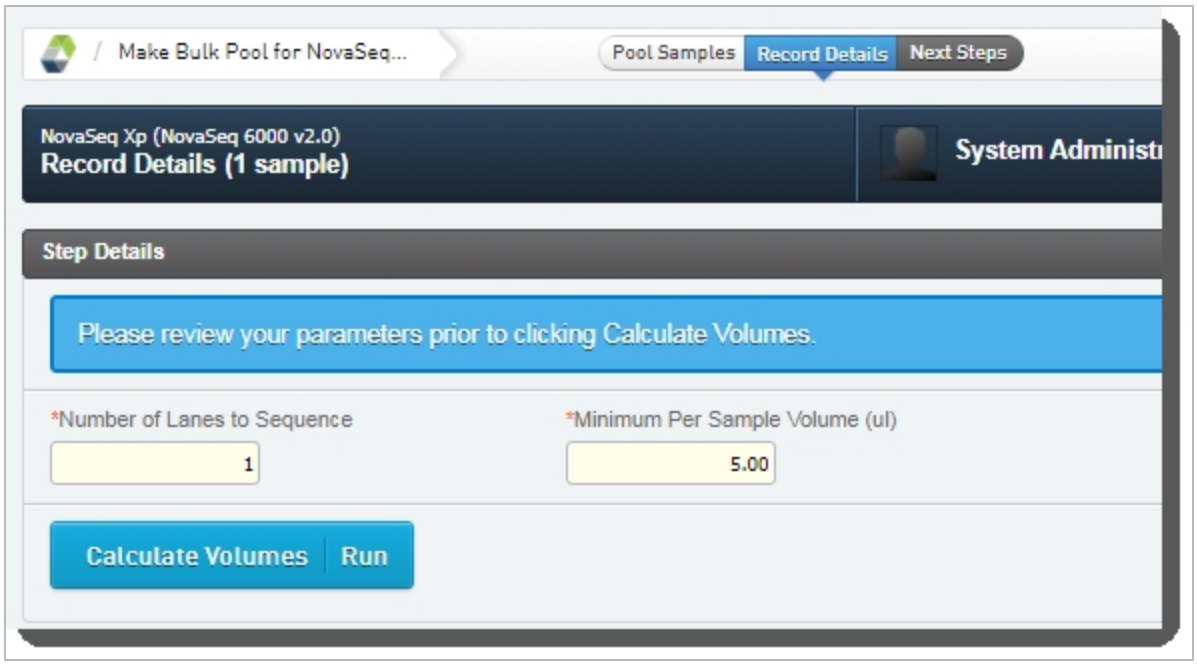
- You can type a name for the pool, or accept the default name - Pool #1.



- Click Record Details.

4. On exit from the Pooling screen, the Validate Inputs Flowcell Type and Single Pool is triggered. The automation checks that: All samples in the pool have been assigned the same Flowcell Type.
    - Only one pool has been created
5. On the Record Details screen, the Step Details area contains two required fields:Number of Lanes to Sequence: The value entered in this field is used in volume calculations, to ensure that volumes are sufficient for the number of times the pool will be sequenced.
    - Minimum Per Sample Volume (ul): The value in this field is used to calculate how much of each sample will be included in the pool. The field is pre-populated with the configured default value - 5 ul, but may be edited if required.

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Note:
Assuming the default Minimum Per Sample Volume (ul) value of 5, for a given batch:
1. If the smallest Per Sample Volume (ul) value is less than 5, the LIMS automatically assigns a value of 5 to the sample's Adjusted Per Sample Volume (ul) field.
2. The LIMS then adjusts the Adjusted Per Sample Volume (ul) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

- In the Sample Details table, you can click on the pool icon to view details on the pool composition.
- Click Calculate Volumes. This triggers the Calculate Volumes automation, which performs volume calculations based on the selected Flowcell Type : Calculates Bulk Pool Volume (ul) value.

– Calculates the Per Sample Volume (ul) value to be added to the pool.
– Calculates the Total Sample Volume (ul) value.
– If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (ul) value.
– Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
– The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains volume information about the samples and RSB buffer to add to the pool. Click on the file to download it and then open it in Excel.

- Click Next Steps. This triggers the Set Next Step automation. This automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol - Dilute, Denature and ExAmp (NovaSeq 6000 v2.0).

6. On the Assign Next Steps screen, the next step for samples is already set to the next step in the workflow - Dilute, Denature and ExAmp (NovaSeq 6000 v2.0).
7. Click Finish Step.

At the end of this step, the pool of samples automatically advances to the Dilute, Denature and ExAmp (NovaSeq 6000 v2.0) step.

2. Run Dilute, Denature and ExAmp (NovaSeq 6000 v2.0) step

1. In Lab View, locate the NovaSeq Xp (NovaSeq 6000 v2.0) protocol. You will see the pool of samples queued for the Dilute, Denature and ExAmp (NovaSeq 6000 v2.0) step. Add the pool to the Ice Bucket.
2. On the Ice Bucket screen, set the number of derivatives to create (these will be placed into the flow cell lanes) and click Begin Work.
3. The Validate Inputs Flowcell Type automation checks that there is only one container input to the step. On entry to the Record Details screen, the Calculate Volumes automation is triggered. This automation sets the following values based on the selected Flowcell Type :NaOH Volume (ul)
    - Tris-HCl Volume (ul)
    - DPX1 Volume (ul)
    - DPX2 Volume (ul)
    - DPX3 Volume (ul)
    - Mastermix per Lane (ul)
    - The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains information about the DPX Mastermix volume, and the volume of Mastermix, NaOH. and Tris-HCl to add per working pool. (Seestep 5, below.)
4. On the Record Details screen, the Reagent Lot Tracking section lets you track the DPX1, DPX2, DPX3, NaOH, Resuspension Buffer, and Tris-HCl reagents used in the step. You will need to add and activate lots for these reagents. To add and activate reagent lots:
    - In a new browser tab, open an additional instance of the LIMS. LIMS v5.0: Navigate to the Consumables configuration screen.

    – LIMS v4.2: Navigate to the Reagents and Controls screen.
        - Select one of the reagent kits listed above (NaOH shown below) and click New Lot.
        - Enter the lot details and click Save.
        - Set the Status of Reagent Lot toggle switch to Active.
        - Repeat this process to add and activate the remaining reagent lots.
        - Close this browser tab and return to your original working tab. Refresh the page.

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5. On the Record Details screen: In the Reagent Lot Trackingsection, select from the active lots displayed in each drop-down list.



   - In the Step Details area, the DPX1, DPX2, and DPX3reagent volume values are already populated. (These values are set by a script and are not editable by the user running the step.)



   - In the Sample Details table: You can click on the pool icon to view details on the working pool composition.

   – The BP Aliquot, Mastermix per lane, NaOH, and Tris-HCl volume values for each working pool are already populated. (These values are set by a script and are not editable by the user running the step.)
   – The working pool number is appended to the bulk pool name. This allows users to quickly identify which working pools are derived from the same bulk pool.

6. In the Files area, click on the Calculation File (CSV) to open it and view details on the DPX Mastermix volume, and the volume of Mastermix, NaOH. and Tris-HCl to add per working pool.
7. Click Next Steps.
8. On the Assign Next Steps screen, the next step is already set to Load to Flowcell (NovaSeq 6000 v2.0).
9. Click Finish Step.

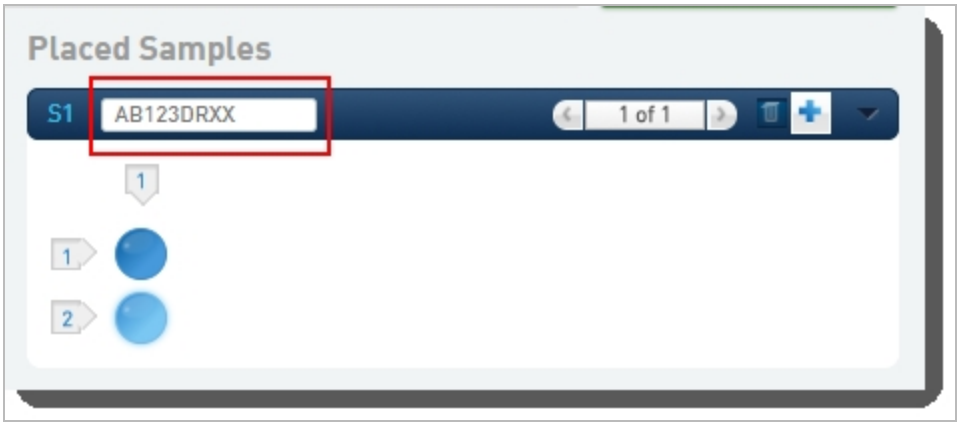
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3. Run Load to Flowcell (NovaSeq 6000 v2.0) step

1. On the Ice Bucket screen: In the Container Options panel, select the appropriate flow cell type from the Destination Containerdrop-down list.



   - Click Begin Work.
2. On the Placement screen: Drag the pool(s) from the left of the screen over into the Placed Samples area on the right.
   - Scan or type the barcode of the flow cell into the Flow Cellfield.

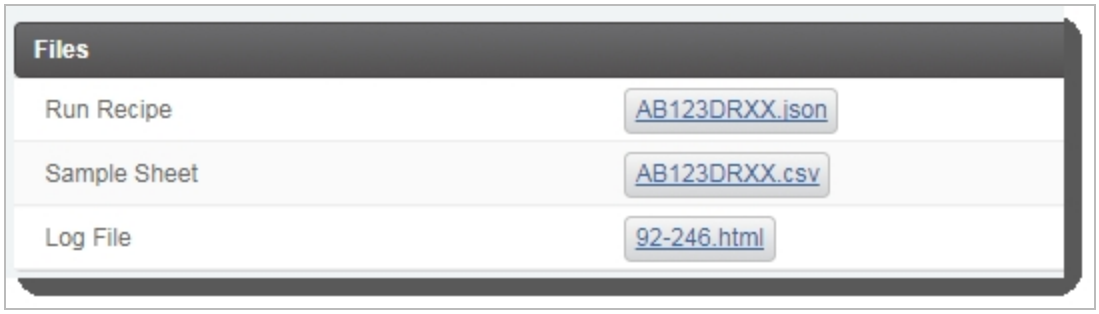


   - Click Record Details. On exit of the Placement screen, the Validate Flowcell Barcode automation validates the container barcode.
3. The fields displayed in the Step Details section are used to generate the sample sheet and run recipe files. Some of these fields are auto-populated and some must be completed by the user (see table below, for details).Fields displayed on Record Details screen of Load to Flowcell (NovaSeq 6000 v2.0) step

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. No spaces.
BaseSpace Sequence Hub Configuration	Select from preset options: Not Used, Run Monitoring Only, or Run Monitoring and Storage

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Field	Value
Workflow	Automatically populated with preset - GenerateFASTQ.
Workflow Type	Select from preset options: No Index, Single Index, Dual Index, or Custom
Index Read 1	Select from preset options: 0, 6, or 8 - or type a value between 0 and 20.
Index Read 2	If Dual Index workflow type selected, once the sample sheet has been generated, Index Read 2 value is automatically populated to match Index Read 1.
Paired End	Select from preset options: True or False
Read 1 Cycles	Select from preset options: 151, 101, or 51 - or type a value between 1 and 151.
Read 2 Cycles	If Paired End = True, once the sample sheet has been generated, Read 2 Cycles value is automatically populated to match Read 1 Cycles.
Use Custom Read 1 Primer	Select if applicable. LIMS v5.0.5 and later: See the *Configuration Update* documentation.
Use Custom Read 2 Primer	Select if applicable. LIMS v5.0.5 and later: See the *Configuration Update* documentation.
Use Custom Index Read 1 Primer	Select if applicable. LIMS v5.0.5 and later: See the *Configuration Update* documentation.
Sample Sheet Path	Automatically populated once the sample sheet has been generated.
Library Tube Barcode	Scan the library tube barcode.

4. Click Generate Sample Sheet and Run Recipe . This triggers the automation script: The sample sheet and JSON files are generated and attached to the placeholders in the Files area of the Record Detailsscreen.
5. 

   - The Sample Sheet Path field is populated with the path to the sample sheet file.
   - If applicable, the Index Read 2 and Read 2 Cycles fields are populated.
6. Click Next Steps. This triggers the Prepare Files for NovaSeq automation which copies the sample sheet and run recipe files to the location specified during installation. The NovaSeq instrument software uses these files to set up the run.
7. On the Assign Next Steps screen, the Next Step for samples is pre-populated with Mark protocol as complete.

8. Click Finish Step.

At this point in the workflow, the user interaction ends. The flow cell is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) step.

Proceed to Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0).

Do not add samples to the Ice Bucket or start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) step. The integration will do this automatically.

Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0)

This protocol contains a single fully-automated step - AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0).

The integration starts the step automatically and data from the run is parsed back into the LIMS. No user interaction is required. However, the user may open and review the various stages of the step in the LIMS.

Review data parsed by AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) step

Once the run is complete, open the step and review the Next Steps screen. Validate the run data parsed into the Step Details and Sample Data tables.

How the integration works

1. When the user initiates a run on the NovaSeq instrument, the NovaSeq Control Software (NVCS) looks for the sample sheet and run recipe (*.json file) on the shared network drive.The NVCS copies the sample sheet to the run directory.
    - The NVCS uses the run recipe to start the sequencing run.
2. When the run starts: The instrument software creates a new run folder on the shared network drive and copies the following files into that folder:RunParameters.xml
    - RunInfo.xml
    - LIMS/ <libraryID>.json file (run recipe)
        - The Real-Time Analysis v3 (RTA3) software creates the InterOp folder.
3. The sequencing service detects the presence of the RunParameters, RunInfo, and JSON files and starts the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) step in the LIMS.
4. As the run progresses, the InterOp data files are filled in.
5. At the end of the run, the NovaSeq creates the CopyComplete.txt file in the run folder, which indicates to the sequencing service that the run has completed and the data files are ready.
6. The NovaSeq copies the run data files to the InterOp folder and the sequencing service triggers the Read InterOp Metrics automation, which reads the files, records the parsed metrics into the LIMS, and finally completes the step in LIMS.

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Parse Run Parameters and Generate Link to Run Folder automation

The Parse Run Parameters and Generate Link to Run Folder automation is automatically triggered on entry to the Record Details screen. This automation parses the RunParameters.xml file attached on the Step Setup screen and populates the step UDFs / custom fields with extracted values. For details on this automation, including how the parsed fields map to the step UDFs / custom fields, see the *NovaSeq 6000 Integration v2.2.1 - User and Configuration Guide*.

Read InterOp Metrics automation

Automatically triggered when the sequencing service detects the CopyComplete.txt file has been created, this automation parses the InterOp data generated by the run.

The read_interop_metrics script records summary metrics into the following global UDFs / custom fields in the LIMS. The script will fail if any field is not configured or cannot be written to.

- Yield PF (Gb) R1
- Yield PF (Gb) R2
- Reads PF (M) R1
- Reads PF (M) R2
- Cluster Density (K/mm^2) R1
- Cluster Density (K/mm^2) R2
- %PF R1
- %PF R2
- % Bases >=Q30 R1
- % Bases >=Q30 R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- % Aligned R1
- % Aligned R
- % Error Rate R1
- % Error Rate R2

For details, see the *User and Configuration Guide*.

Set Next Steps automation

This automation sets the next step for all samples to Mark protocol as complete. For details, see the *User and Configuration Guide*.

Troubleshooting

If an automation trigger does not appear to run its corresponding scripts, see the following articles:

- *Troubleshooting Automated Informatics* in the Clarity LIMS documentation, Automated Informatics section
- *Troubleshooting EPP / Automation* in the API > Clarity LIMS API Overview > API Advanced Topics section

If an error occurs that does not provide direction on how to proceed, complete the following steps:

1. Confirm the version of the installed Illumina NovaSeq Integration Package by running the following command:

```
rpm -qa | grep -i novaseq
```

   - For an on-premise installation, run the command on the LIMS server command line. This will retrieve the version of both RPMs installed.
   - For a Software as a Service (SaaS) installation, run the command on your local server installation. This will retrieve the version of the remote extensions RPM installed.

2. If the error is related to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) step, there are two places to check for log file information: If the step does not start, check the NovaSeqIntegrator.log log file written to:

```
/opt/gls/clarity/extensions/novaseq/SequencingService/NovaSeqIntegrator.log
```

   - This log file will be written to this path wherever the remote extensions RPM was installed.
   - If the step starts but does not complete, open the step in the LIMS (it can be found via a search). On the Record Details screen, the log file will be attached to a placeholder called Log File. If you are unable to reach the Record Details screen (for example, if the step stopped execution on the Step Setup screen), or if the file does not contain an error, review the NovaSeqIntegrator.log described above.

3. Contact the BaseSpace Clarity LIMS support team, supplying the relevant information from the troubleshooting steps already performed.

Additional troubleshooting information is provided on the Illumina Integrations FAQ page.

Illumina NovaSeq 6000 Integration v2.2.1 Release Notes

Version: 2.2.1 Compatibility:

- BaseSpace Clarity LIMS v4.2 or later, though not compatible with v6.0 and later

- NGS Extensions Package v5.15 or later
- BaseSpaceLIMS-AutomationWorker 0.1.0 (LIMS v4.2) or v1.0 (LIMS v5.0)

Illumina NovaSeq Software Compatibility: NovaSeq Control Software (NVCS) v1.3.0

Features to note for this release Maintainsintegrity of sample information throughout the workflow

- Now Support S1 in addition to S2, and S4 flow cells per sample

Known issues

There are no known issues for this release.

Documentation

For detailed information related to this release, see the latest documentation in the Illumina NovaSeq Integration v2 section.

NovaSeq Integration v2.2.1 Feature Demo Video

Illumina NovaSeq 6000 Integration v2.2 Release Notes

Version: 2.2 Compatibility:

- BaseSpace Clarity LIMS v4.2 or later, though not compatible with v6.0 and later
- NGS Extensions Package v5.15 or later
- BaseSpaceLIMS-AutomationWorker 0.1.0 (LIMS v4.2) or v1.0 (LIMS v5.0)

Illumina NovaSeq Software Compatibility: NovaSeq Control Software (NVCS) v1.3.0

Features to note for this release

This release of the BaseSpace Clarity LIMS integration with the Illumina NovaSeq 6000 system includes updated configuration to expose the NovaSeq Xp workflow. Users that upgrade to or perform a clean installation of this version will have:

- Run mode flexibilitySample-based choice of the run mode: NovaSeq Standard using the library tube barcode or NovaSeq Xp using the flow cell loading dock
- An updated workflow with support for:S1*, S2, and S4 flow cells per sample
  - Different applications on the same flow cell
  - Sample and reagent calculations based on flow cell

  - Creation of an output file for use with liquid handling robots
- A run recipe (*.json file) that interacts with the latest NovaSeq 6000 instrument software
- A sample sheet compatible with BaseSpace Sequence Hub and bcl2fastq v2.19
- Automatic tracking of the sequencing run and parsing of run metrics per lane and per flow cell

* S1 flow cell will be released in the near future

Known issues

There are no known issues for this release.

Documentation

For detailed information related to this release, see the following documentation:

- Illumina NovaSeq 6000 Integration - Installation Guideline (SaaS)
- Illumina NovaSeq 6000 Integration - User and Configuration Guide
- Illumina NovaSeq 6000 Integration - Validation and Troubleshooting Guide

Configuration modifications when upgrading from NovaSeq 2.1 or 2.2 to NovaSeq 2.2.1 and NovaSeq 2.2.2

This document is provided for customers who have already installed the Illumina NovaSeq 6000 Integration v2.1or v2.2 packages.

The document shows how to make some minor configuration changes to the NovaSeq 6000 v2.0 workflow to the latest updates.

Note:
System Administrator permissions are required to make the configuration changes described below.

Part 1: Enabling NovaSeq Xp protocol

Part 1 should be done only when upgrading from Illumina NovaSeq 6000 Integration v2.1 to Illumina NovaSeq 6000 Integration v2.2.1 and v2.2.2

Steps required - LIMS v5

To activate the NovaSeq Xp (NovaSeq 6000 v2.0) protocol for use in the NovaSeq 6000 v2.0 workflow, the following steps are required:

1. Display NovaSeq Xp (NovaSeq 6000 v2.0) protocol in Lab View.
2. Configure the Loading Workflow Type global field as follows: Add NovaSeq Xp dropdown list item
    - Reorder dropdown list items

- Remove default field value (NovaSeq Standard) configured for NovaSeq v2.1

1. Display NovaSeq Xp (NovaSeq 6000 v2.0) protocol in Lab View

1. On the Configuration tab, click Lab Work.
2. In the Protocols list, select the NovaSeq Xp (NovaSeq 6000 v2.0) protocol.
3. In the Protocol Settings section, set the Show in Lab View? toggle switch to Yes.
4. Click Save.

2. Configure Loading Workflow Type global field

1. On the Configuration tab, click Custom Fields.
2. On the Global Fields tab, expand the Derived Samplefield list.
3. Select the Loading Workflow Typefield. In the Field Details area on the right, the dropdown items currently configured for the field are listed under Additional Options.
4. To add a new dropdown item, click the + button, and then type NovaSeq Xpin the text box that displays. As you cannot reorder list items by dragging and dropping them, you now need to add a new item to the end of the list - for the [Remove from workflow] option. You can then delete the existing item.
5. Click inside the [Remove from workflow] text box. Click and drag to select all of the text, and then click Ctrl + Cto copy it to the Clipboard.
6. Click the + button to add another new item. Click inside the text box and then press Ctrl + V to paste the contents of the Clipboard.Because the LIMS does not allow duplicate dropdown list items, both items will be highlighted in red.
7. Click the X button to the right of the original dropdown item to delete it.
8. On the NovaSeq Standard list item, set the Set as Default? toggle switch to No.
9. Click Save.

Steps required - LIMS v4.2

To activate the NovaSeq Xp (NovaSeq 6000 v2.0) protocol for use in the NovaSeq 6000 v2.0 workflow, the following steps are required:

1. Display NovaSeq Xp (NovaSeq 6000 v2.0) protocol in Lab View.
2. Configure the Loading Workflow Type UDF as follows: Add NovaSeq Xp preset value
    - Reorder preset values
    - Remove default preset value configured for NovaSeq v2.1

1. Display NovaSeq Xp (NovaSeq 6000 v2.0) protocol in Lab View

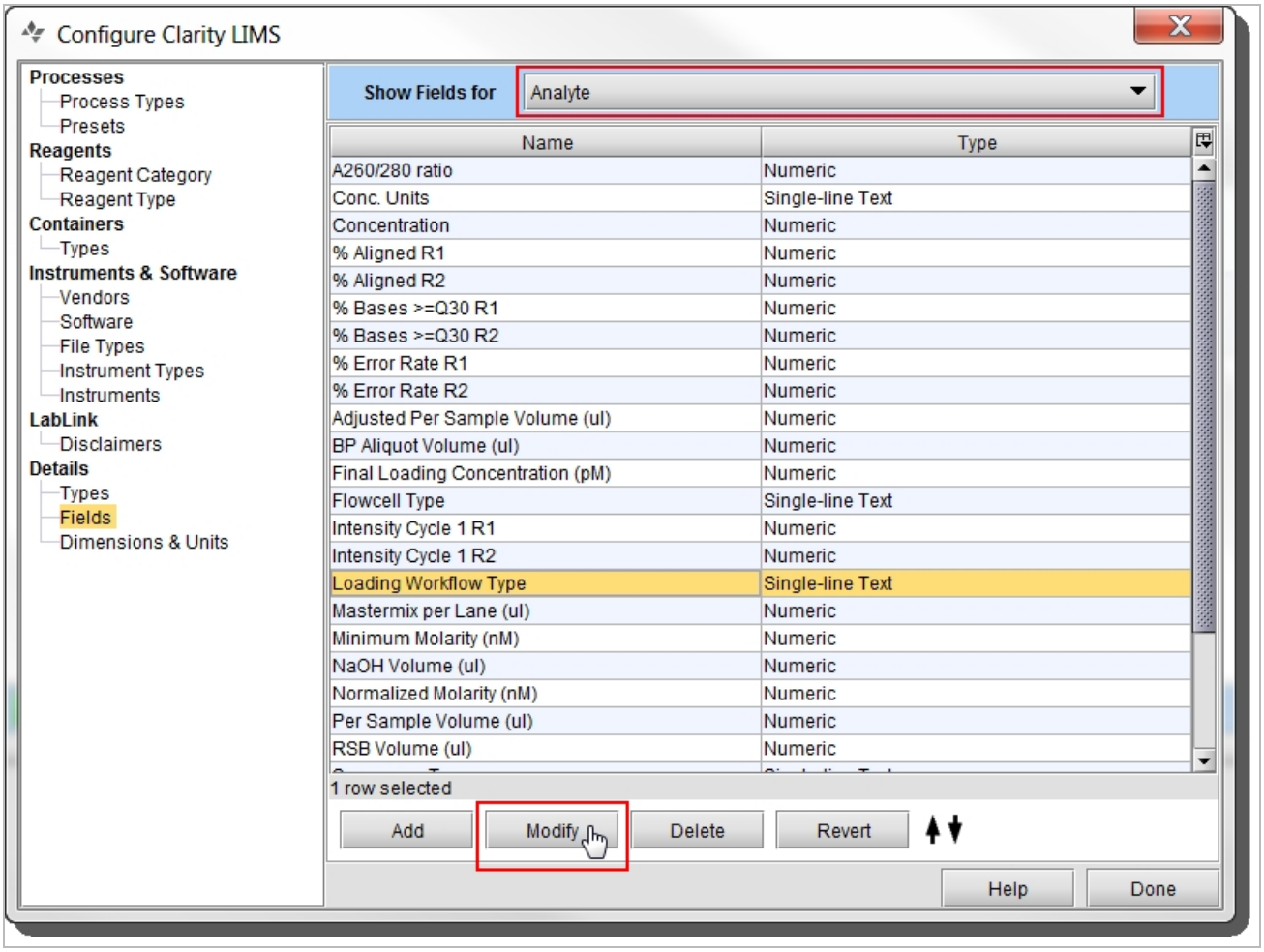
1. In the BaseSpace Clarity LIMS interface, click Configure > Lab.
2. On the Protocols tab, hover over the NovaSeq Xp (NovaSeq 6000 v2.0) protocol and click the Editbutton that displays.
3. On the NovaSeq Xp (NovaSeq 6000 v2.0) configuration screen, clear the Hiddencheck box.

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4. Click Save Protocol.

2. Configure Loading Workflow Type UDF

1. In the Operations Interface, on the Configuration menu, click Details > Fields.
2. In the Show Fields for drop-down list, select Analyte.
3. Select the Loading Workflow Type field and click Modify.



In the Modify Field dialog, the Preset Values tab lists the preset values currently configured for the field. These are the values that display in the Loading Workflow Type drop-down list.

4. To add a new value, click the Add button, and then type NovaSeq Xp in the text box that displays.
5. Use the arrow button to move the new value up so that it displays directly below NovaSeq Standard.
6. On the Field Constraints tab, clear the Use first Preset Value as the default valuecheck box.
7. Click Save.

Results

The configuration steps described above result in the following changes in the LIMS.

- In Lab View The NovaSeq Xp (NovaSeq 6000 v2.0) protocol displays in the Available Workprotocol list.
- When running the Define Run Format (NovaSeq 6000 v2.0) step On the Record Details screen, in the Sample Details table, NovaSeq Xp displays in the Loading Workflow Typedrop-down list. Samples with Loading Workflow Type set to NovaSeq Xp will be routed to the NovaSeq Xp (NovaSeq 6000 v2.0) protocol.

Part 2: Modifying the Automation for the S1 Flowcell

Part 2 should be done when upgrading from either Illumina NovaSeq 6000 Integration v2.1 or Illumina NovaSeq 6000 Integration v2.2.

Steps required - LIMS v5

1. On the Configuration tab, click Automation.
2. On the Step Automation tab, select Calculate Volumes for Make Bulk Pool for NovaSeq Standard.
3. Delete the existing automation and replace with the following: *bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} -t true -h false -log {compoundOutputFileLuid1} \script:evaluateDynamicExpression \-exp 'step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: + 1' \script:evaluateDynamicExpression \-exp 'if (input.::Flowcell Type:: == ::S1::) { step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 100 + 30 } ; if (input.::Flowcell Type:: == ::S2::) { step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 150 + 30 } ; if (input.::Flowcell Type:: == ::S4::) { step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 310 + 30 } ; input.::Per Sample Volume (ul):: = (((input.::Final Loading Concentration (pM):: * 5 / 1000) / input.::Normalized Molarity (nM)::) * step.::Bulk Pool Volume (ul)::) / step.::Number of Samples in Pool::' \&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/novaseq/novaseq-extensions.jar script:calculate_adjusted_per_sample_volume -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} \&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} -t true -h false -log {compoundOutputFileLuid1} \script:evaluateDynamicExpression \-exp 'step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: + input.::Adjusted Per Sample Volume (ul)::' \script:evaluateDynamicExpression \-exp 'if (step.::Total Sample Volume (ul):: >= step.::Bulk Pool Volume (ul)::) {output.::RSB Volume (ul):: = 0} else {output.::RSB Volume (ul):: = step.::Bulk Pool Volume (ul):: - step.::Total Sample Volume (ul)::} ; output.::Flowcell Type:: = input.::Flowcell Type:: ; output.::Loading Workflow Type:: = input.::Loading Workflow Type:: ; if (input.::Flowcell Type:: == ::S1::) { output.::Volume of Pool to Denature (ul):: = 100 ; output.::NaOH Volume (ul):: = 25 ; output.::Tris-HCl Volume (ul):: = 25 } ; if (input.::Flowcell Type:: == ::S2::) {output.::Volume of Pool to Denature (ul):: = 150 ; output.::NaOH Volume (ul):: = 37.50 ; output.::Tris-HCl Volume (ul):: = 37.50 } ; ; if (input.::Flowcell Type:: == ::S4::) { output.::Volume of Pool to Denature (ul):: = 310 ; output.::NaOH Volume (ul):: = 77.50 ; output.::Tris-HCl Volume (ul):: = 77.50 }' \&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-*

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common/v5/EPP/DriverFileGenerator.jar -i {processURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} \script:driver_file_generator \-t /opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Standard_Bulk_Pool1.csv \-o 1.csv \script:driver_file_generator \-t /opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Standard_Bulk_Pool2.csv \-o 2.csv \script:driver_file_generator \-t /opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Standard_Bulk_Pool3.csv \-o 3.csv \&& cat 1.csv 2.csv 3.csv > {compoundOutputFileLuid0}.csv"

4. Click Save.

Steps required - LIMS v4.2

1. In the Operations Interface, on the Configuration menu, click Process > Process Types.
2. In the drop-down list, look for the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0) and click Modify.
3. Go to the External Programs tab, select the Calculate Volumes automation and click Modify.
4. Delete the existing automation and replace with the following: *bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} -t true -h false -log {compoundOutputFileLuid1} \script:evaluateDynamicExpression \-exp 'step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: + 1' \script:evaluateDynamicExpression \-exp 'if (input.::Flowcell Type:: == ::S1::) { step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 100 + 30 } ; if (input.::Flowcell Type:: == ::S2::) { step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 150 + 30 } ; if (input.::Flowcell Type:: == ::S4::) { step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 310 + 30 } ; input.::Per Sample Volume (ul):: = (((input.::Final Loading Concentration (pM):: * 5 / 1000) / input.::Normalized Molarity (nM)::) * step.::Bulk Pool Volume (ul)::) / step.::Number of Samples in Pool::' \&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/novaseq/novaseq-extensions.jar script:calculate_adjusted_per_sample_volume -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} \&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} -t true -h false -log {compoundOutputFileLuid1} \script:evaluateDynamicExpression \-exp 'step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: + input.::Adjusted Per Sample Volume (ul)::' \script:evaluateDynamicExpression \-exp 'if (step.::Total Sample Volume (ul):: >= step.::Bulk Pool Volume (ul)::) {output.::RSB Volume (ul):: = 0} else {output.::RSB Volume (ul):: = step.::Bulk Pool Volume (ul):: - step.::Total Sample Volume (ul)::} ; output.::Flowcell Type:: = input.::Flowcell Type:: ; output.::Loading Workflow Type:: = input.::Loading Workflow Type:: ; if (input.::Flowcell Type:: == ::S1::) { output.::Volume of Pool to Denature (ul):: = 100 ; output.::NaOH Volume (ul):: = 25 ; output.::Tris-HCl Volume (ul):: = 25 } ; if (input.::Flowcell Type:: == ::S2::) {output.::Volume of Pool to Denature (ul):: = 150 ; output.::NaOH Volume (ul):: = 37.50 ; output.::Tris-HCl Volume (ul):: = 37.50 } ; ; if (input.::Flowcell Type:: == ::S4::) { output.::Volume of Pool to Denature (ul):: = 310 ; output.::NaOH Volume (ul):: = 77.50 ; output.::Tris-HCl Volume (ul):: = 77.50 }' \&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/DriverFileGenerator.jar -i {processURI:v2} -u {username} -p {password} -l*

For Research Use Only. Not for use in diagnostic procedures.

{compoundOutputFileLuid1} \script:driver_file_generator \-t /opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Standard_Bulk_Pool1.csv \-o 1.csv \script:driver_file_generator \-t /opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Standard_Bulk_Pool2.csv \-o 2.csv \script:driver_file_generator \-t /opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Standard_Bulk_Pool3.csv \-o 3.csv \&& cat 1.csv 2.csv 3.csv > {compoundOutputFileLuid0}.csv"

5. Click Save, Save, and Done.

Illumina NovaSeq 6000 Integration v2.1 Configuration and User Interaction

Introduction

The Illumina NovaSeq 6000 Integration Package v2.1 supports the integration of BaseSpace Clarity LIMS to Illumina NovaSeq 6000 sequencers.

This *User and Configuration Guide* describes the integration and includes information about the preconfigured protocols, steps, and automations; installed components; and configuration requirements, rules, and constraints.

For instructions on validating and troubleshooting the Illumina NovaSeq 6000 Integration, see the *NovaSeq 6000 Integration v2.1 - Validation and Troubleshooting Guide*.

Overview

The integration includes the following functionality:

- Preconfigured NovaSeq 6000 v2.0 workflow that maps to lab protocols and instrument runs.
- Preconfigured NovaSeq Standard (NovaSeq 6000 v2.0) protocol that supports the loading of pooled libraries into a library tube.
- Preconfigured NovaSeq Xp (NovaSeq 6000 v2.0) protocol (hidden) that allows for the support of future functionality. For details, see Note below.
- Automated generation of a sample sheet for use with bcl2fastq2 v2.19 analysis software.
- Automatic generation of run recipe file (JSON format) that is automatically uploaded to the sequencing instrument and used to set up and initiate the run.
- Automated tracking of the NovaSeq sequencing run and parsing of run statistics (per run per lane) into the LIMS, including:Progress and metrics of sequencing run
  - Per-instrument sequencing runs (tracked as part of the Run ID field value)
  - Sequencing run parameters
  - Real Time Analysis v3 (RTA3) run directory location and other run specific information

- Preconfigured NovaSeq Validation Library Prep (NovaSeq 6000 v2.0) workflow used for validation purposes only. The workflow contains a single-step protocol that models the library prep required to produce normalized libraries that are ready for the NovaSeq 6000 v2.0 workflow. For details, see the *NovaSeq 6000 Integration v2.1 - Validation and Troubleshooting Guide*.

Information on these features is provided in the following sections.

Note:
The NovaSeq Xp (NovaSeq 6000 v2.0) protocol will support individual lane loading on the NovaSeq in a future release. In NovaSeq 6000 Integration Package v2.1, the protocol is not displayed to users running samples through the LIMS; however, administrator users will see it in the Configuration screen.

Prerequisites and assumptions

It is assumed that samples enter the NovaSeq 6000 v2.0 workflow as normalized libaries. That is, before they are assigned to the workflow:

- Samples have been accessioned into the LIMS.
- Samples have been run through QC and library prep.
- Samples have been normalized, and the value is captured in a field called Normalized Molarity (nM).

For details on sample accessioning, see the following articles in the LIMS Documentation (Samples section):

- *Sample Accessioning*
- *Using Sample Lists to Upload and Modify Samples*

Samples can be assigned to the NovaSeq 6000 v2.0 workflow automatically (using a routing script), or manually from the Projects and Samples dashboard (see the *Assigning Samples to Workflows* article).

Workflows, protocols, and steps

The Illumina NovaSeq 6000 Integration Package v2.1 includes two workflows:

- NovaSeq 6000 v2.0
- NovaSeq Validation Library Prep (NovaSeq 6000 v2.0)

Table 1 lists and describes the protocols and steps included in these workflows.

Table 1: Illumina NovaSeq 6000 Integration Package v2.1 protocols and steps

NovaSeq Validation Library Prep (NovaSeq 6000 v2.0) workflow

Protocol name	Protocol purpose	Steps included in protocol

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NovaSeq Validation Library Prep (NovaSeq 6000 v2.0) workflow

NovaSeq Validation Library Prep (NovaSeq 6000 v2.0)	Included for validation purposes only, this protocol models the library prep steps required to advance samples to the Run Format (NovaSeq 6000 v2.0) protocol. The protocol contains a single step - NovaSeq Validation Library Prep (NovaSeq 6000 v2.0). At the end of this step, a routing script sends the samples to the first step of the NovaSeq 6000 v2.0 workflow - Define Run Format (NovaSeq 6000 v2.0).	1. NovaSeq Validation Library Prep (NovaSeq 6000 v2.0)

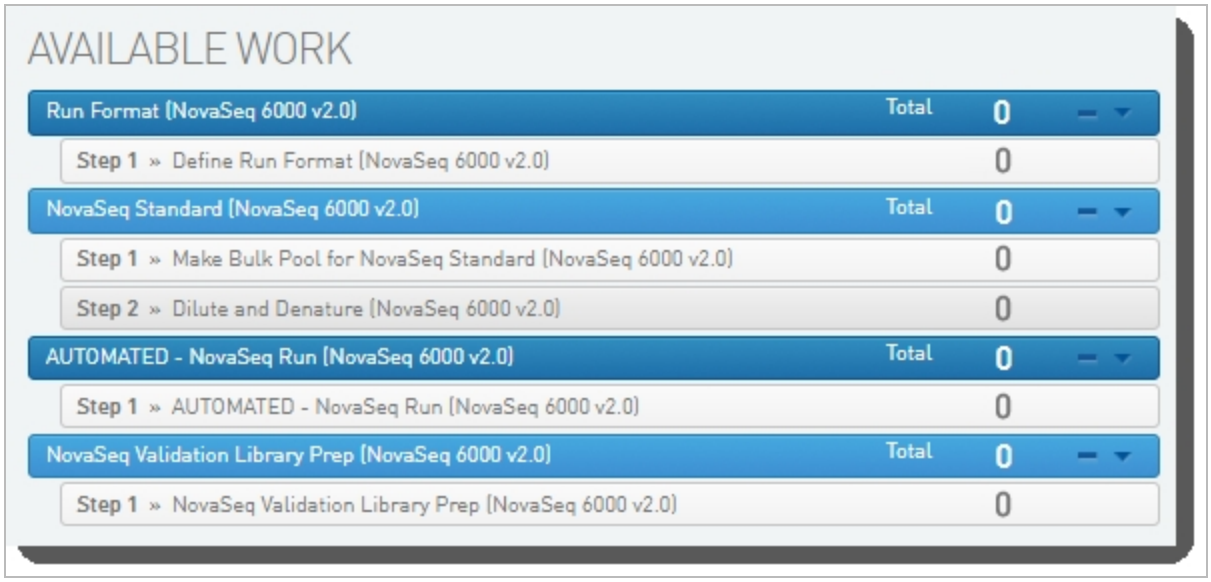
NovaSeq 6000 v2.0 workflow

Protocol name	Protocol purpose	Steps included in protocol
Run Format (NovaSeq 6000 v2.0)	Allows for the assignment of per sample values for Loading Workflow Type, Normalized Molarity, Flowcell Type, and Final Loading Concentration (pM): • Loading Workflow Type: As this release only supports the standard workflow, this field is pre-populated by the LIMS with the value NovaSeq Standard. • Normalized Molarity: User enters a value for each sample. • Flowcell Type: User selects from options S1, S2, and S4. • Final Loading Concentration (pM): User selects from the options 225 (PCR-free workflows) or400 (Nano workflows), or may enter a different value. Compares each sample's Normalized Molarity value with the Minimum Molarity value. Routing script sends samples to the NovaSeq Standard protocol, or removes them from the workflow if Normalized Molarity is less than Minimum Molarity.	1. Define Run Format (NovaSeq 6000 v2.0
NovaSeq Standard (NovaSeq 6000 v2.0	Samples are pooled and added to the library tube in preparation for the NovaSeq run. Routing script sends library tube to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) protocol.	1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0) 2. Dilute and Denature (NovaSeq 6000 v2.0)

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NovaSeq Validation Library Prep (NovaSeq 6000 v2.0) workflow		
NovaSeq Xp (NovaSeq 6000 v2.0)	*In this release, this protocol is hidden from Lab View and the user cannot route samples to it. See Noteabove.*	
AUTOMATED - NovaSeq Run (NovaSeq6000 v2.0)	Samples complete the workflow by going through this protocol. Generation of the samplesheet and the JSON file is dependent on the Loading Workflow Type. Note that in this release, the only supported workflow type is NovaSeq Standard.) This protocol contains 1 fully-automated step. The user must not add samples to the Ice Bucket or start the step. The integration will do this automatically.	1. AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0)

The following image show the protocols and their steps displayed in the LIMS Lab View.



Validation workflow

The NovaSeq Validation Library Prep (NovaSeq 6000 v2.0) workflow allows for validation of the system after installation is complete. For details, see the *NovaSeq 6000 Integration v2.1 - Validation and Troubleshooting Guide*.

Step automations

Table 2 lists the step automations included in the NovaSeq 6000 v2.0 workflow. Automation details are provided in the sections following the table.

For details on the validation workflow, see the *NovaSeq 6000 Integration v2.1 - Validation and Troubleshooting Guide*.

Table 2: NovaSeq 6000 v2.0 workflow step automations

Protocol	Step	Automation
1. Run Format (NovaSeq 6000 v2.0)	1. Define Run Format (NovaSeq 6000 v2.0)	• Routing Script • Set Next Step
2. NovaSeq Standard (NovaSeq 6000 v2.0)	1. Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0)	• Calculate Volumes • Set Next Step • Validate Inputs Flowcell Type and Single Pool • Validate Unique Indexes *Not used. This functionality is handled by the core LIMS configuration for pooling.*
	2. Dilute and Denature (NovaSeq 6000 v2.0)	• Generate Sample Sheet and Run Recipe • Prepare Files for NovaSeq • Routing Script • Validate Library Tube Barcode • Validate Single Input
3. NovaSeq Xp (NovaSeq 6000 v2.0)	*In this release, this protocol is hidden from Lab View and the user cannot route samples to it. See Noteabove.*	
4. AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0)	1. AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0)	• Parse Run Parameters and Generate Link to Run Folder • Read InterOp Metrics • Set Next Steps

The sections below describe the configuration installed with the Illumina NovaSeq 6000 Integration.

Note:
The Illumina NovaSeq 6000 Integration Package v2.1 supports BaseSpace Clarity LIMS v4.2 and v5.0.
As of LIMS v5, the term user-defined field (UDF) has been replaced with custom field in the user interface (the API resource is still called udf). There are two types of custom fields:

- Master step fields - configured on master steps. These fields only apply to the master step on which they are configured, and the steps derived from those master steps.
- Global fields - configured on entities such as submitted sample, derived sample, measurement, etc. These fields apply to the whole LIMS system.

Protocol 1: Run Format (NovaSeq 6000 v2.0)

This protocol sets the Loading Workflow Type and allows the user to choose the appropriate Flowcell Type and Final Loading Concentration(pM). At the end of the protocol, a routing script sends the normalized libraries to the NovaSeq Standard (NovaSeq 6000 v2.0) protocol.

This protocol contains one step: Step 1: Define Run Format (NovaSeq 6000 v2.0), described below.

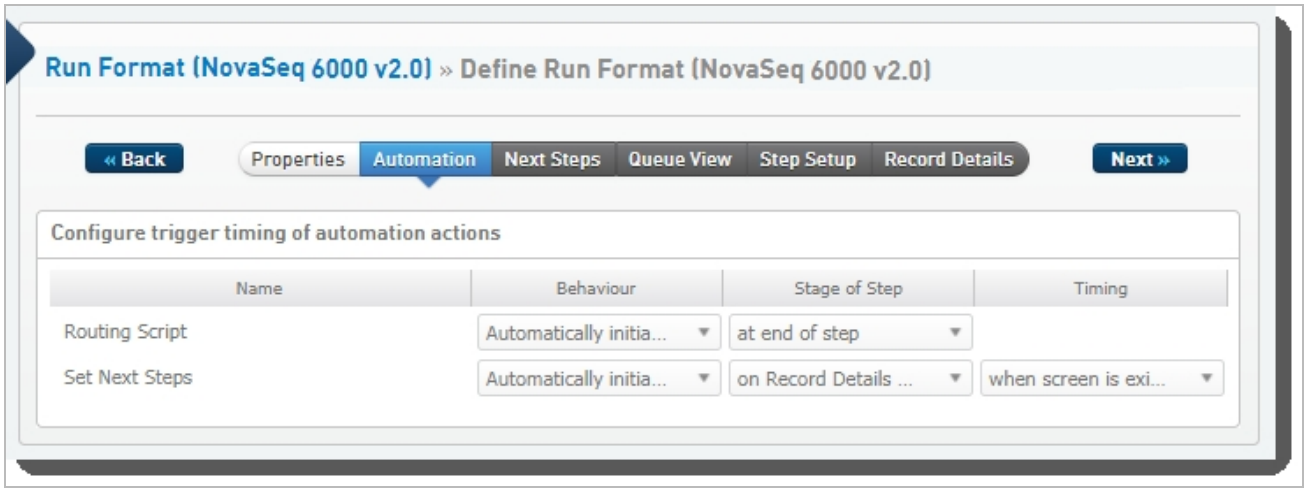
Step 1: Define Run Format (NovaSeq 6000 v2.0)

Step input: NTP (normalized libraries)

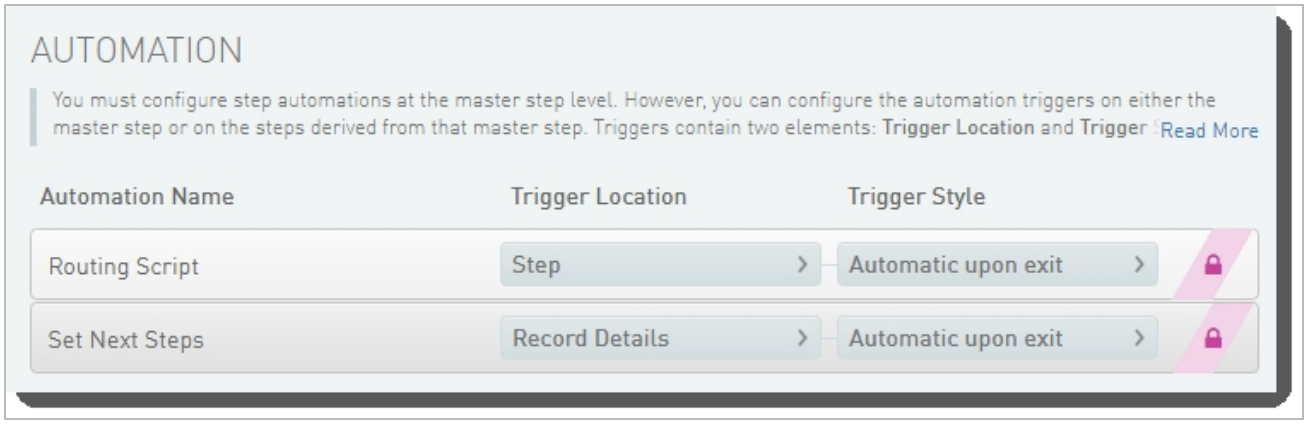
Step output: None

The following automations are configured on this step:

- Routing Script
- Set Next Steps



Define Run Format (NovaSeq 6000 v2.0) automation configuration - LIMS v4.2



Define Run Format (NovaSeq 6000 v2.0) automation configuration - LIMS v5

These automations are described below, in the order in which they are triggered at run time

Set Next Steps

Automatically triggered on exit of the Record Details screen, this automation does the following:

- Sets the next step for samples to REMOVE:

```
nextStep = ::REMOVE::
```

- Calculates the Minimum Molarity using the following formula:

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```
input.::Minimum Molarity (nM):: = (5 * input.::Final Loading Concentration
(pM)::)/1000
```

- Checks Normalized Molarity value. For samples with no Normalized Molarity value (i.e., empty value, not including 0), generates an error message informing the user that the field cannot be empty:

```
if (!input.hasValue(::Normalized Molarity (nM)::)) { fail(::The Normalized
Molarity cannot be empty.::) ; }
```

- Compares each sample's Normalized Molarity value with the Minimum Molarity value.

  If Normalized Molarity value is lower than the Minimum Molarity value, sets the sample's Loading Workflow Type to [Remove from workflow] and records a message in the Warning field for the sample:

```
else if (input.::Normalized Molarity (nM):: < input.::Minimum Molarity (nM)::) {
input.::Warning:: = ::The Normalized Molarity is too low.:: ; input.::Loading
Workflow Type:: = ::[Remove from workflow]:: ; } else { input.::Warning:: =
::n/a:: }
```

  At this point the user has two options:

  - Correct the Normalized Molarity value on the Record Details screen. User will also need to edit the Loading Workflow Type field and set it to NovaSeq Standard.
  - Complete the protocol without correcting the Normalized Molarity value. In this case, those samples will be removed from the workflow.

Routing Script

Automatically triggered on exit of the step, this automation invokes the changeWorkflow script, which routes step inputs appropriately.

- Samples with Loading Workflow Type field value = NovaSeq Standard are routed to the NovaSeq 6000 v2.0 workflow and queued for the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0) step.

Default automation command line:

```
bash -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -u {username} -p {password} -i {stepURI:v2} -l
{compoundOutputFileLuid0} script:changeWorkflow \\--FIELD_NAME 'Loading Workflow
Type' \--FIELD_VALUE 'NovaSeq Standard' \--WORKFLOW 'NovaSeq 6000 v2.0' \--STEP
'Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0)' \--INPUTS_OR_OUTPUTS
'INPUTS' \\--FIELD_NAME 'Loading Workflow Type' \--FIELD_VALUE 'NovaSeq Xp' \--
WORKFLOW 'NovaSeq 6000 v2.0' \--STEP 'Make Bulk Pool for NovaSeq Xp (NovaSeq 6000
v2.0)' \--INPUTS_OR_OUTPUTS 'INPUT
```

Step UDFs/custom fields

There are two fields defined on the Define Run Format (NovaSeq 6000 v2.0) step.

These fields are shown below in the BaseSpace Clarity LIMS v5 Configuration area (Custom Fields > Master Step Fields tab). See Table 3 for LIMS v4.2 and LIMS v5 field configuration details.

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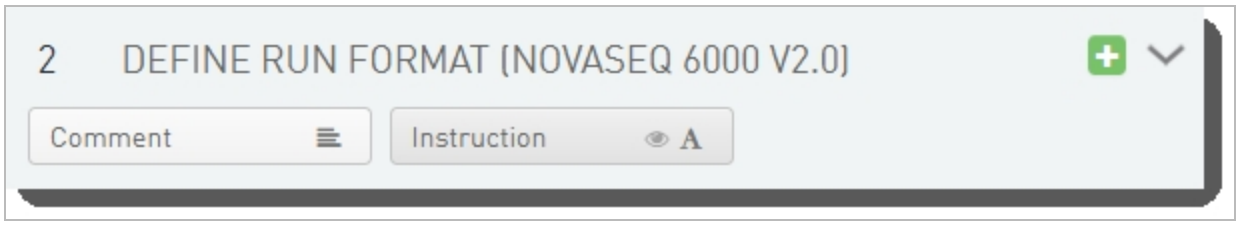


Table 3: Dilute and Denature (NovaSeq 6000 v2.0) step UDF / custom field configuration

Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
Comment	Multi-line Text	• Required: Yes • Read Only: No	None
Instruction	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: Yes	• Default: Add Flowcell Type and Loading Workflow Type below

Global UDFs/custom fields

Table 4 lists the global UDFs / custom fields that are configured in the LIMS to support the Define Run Format (NovaSeq 6000 v2.0) step.

Table 4: Global UDF / custom field configuration

Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
Configured on Analyte in LIMS v4.2 and Derived Samplein LIMS v5:			
Adjusted Per Sample Volume (ul)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Final Loading Concentration (pM)	Numeric (v4.2) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 225 • 400 • Decimal Places Displayed: 0
Flowcell Type	Single-line Text (v4.2) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• S1 • S2 • S4
Loading Workflow Type	Single-line Text (v4.2) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • [Remove from workflow]

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Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
Minimum Molarity (nM)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2
Normalized Molarity (nM)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2
Per Sample Volume (ul)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2

User interaction

1. On the Record Details screen, the Sample Details table contains the following fields that must be completed (values can vary across samples):Loading Workflow Type: As this release of the integration only supports the standard workflow, this field is pre-populated with the value NovaSeq Standard.
    - Flowcell Type: User selects S1, S2, or S4 from the drop-down list.
    - Final Loading Concentration (pM): User selects from the two preset options - 225 (for PCR-free workflows) or400 (for Nano workflows), or may enter a different value.
    - Normalized Molarity: User enters a value for each sample.
    - User clicks Next Steps. This triggers the Next Steps automation, which does the following: Sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value, and requires it in order to successfully advance samples to the next step.
    - Calculates the Minimum Molarity.
    - Checks Normalized Molarity value. For samples with no Normalized Molarity value (i.e., empty value, not including 0), generates an error message informing the user that the field cannot be empty.
    - Compares each sample's Normalized Molarity value with the Minimum Molarity value.
2. On the Assign Next Steps screen:
    - In the Sample Details table, the Next Step for all samples is pre-populated with Remove from workflow - regardless of the Loading Workflow Type The user must not change this value! If Next Step is not set to Remove from workflow, the routing script will not be able to route samples correctly.
    - For samples whose Normalized Molarity value was found to be lower than the Minimum Molarity value, the Loading Workflow Type is set to Remove from workflow and a message is recorded in the Warning field for the sample. At this point the user has two options:

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- Return to the Record Details screen and adjust the Normalized Molarity value so that it equals or exceeds the Minimum Molarity value. User will also need to set the Loading Workflow Type to NovaSeq Standard.
- Complete the protocol without correcting the Normalized Molarity value. In this case, the samples in question will be removed from the LIMS workflow.

3. User clicks Finish Step. The Routing Script automation is triggered:
    - Samples whose Loading Workflow Type is set to Remove from workflow (i.e., whereNormalized Molarity value is lower than the Minimum Molarity) are removed from the LIMS workflow.
    - Samples whose Loading Workflow Type is set to NovaSeq Standard are routed to the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0) step. This is the first of two steps in the NovaSeq Standard (NovaSeq 6000 v2.0) protocol.

Protocol 2: NovaSeq Standard (NovaSeq 6000 v2.0)

Samples are routed to this protocol if their Loading Workflow Type value is set to NovaSeq Standard. Samples are pooled and added to a library tube in preparation for the NovaSeq run.

At the end of this protocol, a routing script sends the library tube to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) protocol.

This protocol contains two steps, described below:

- Step 1: Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0)
- Step 2: Dilute and Denature (NovaSeq 6000 v2.0)

Step 1: Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0)

In this step, user manually places libraries into a single pool. Resuspension buffer and reagents are added.

Note:
User must only create one pool per step.

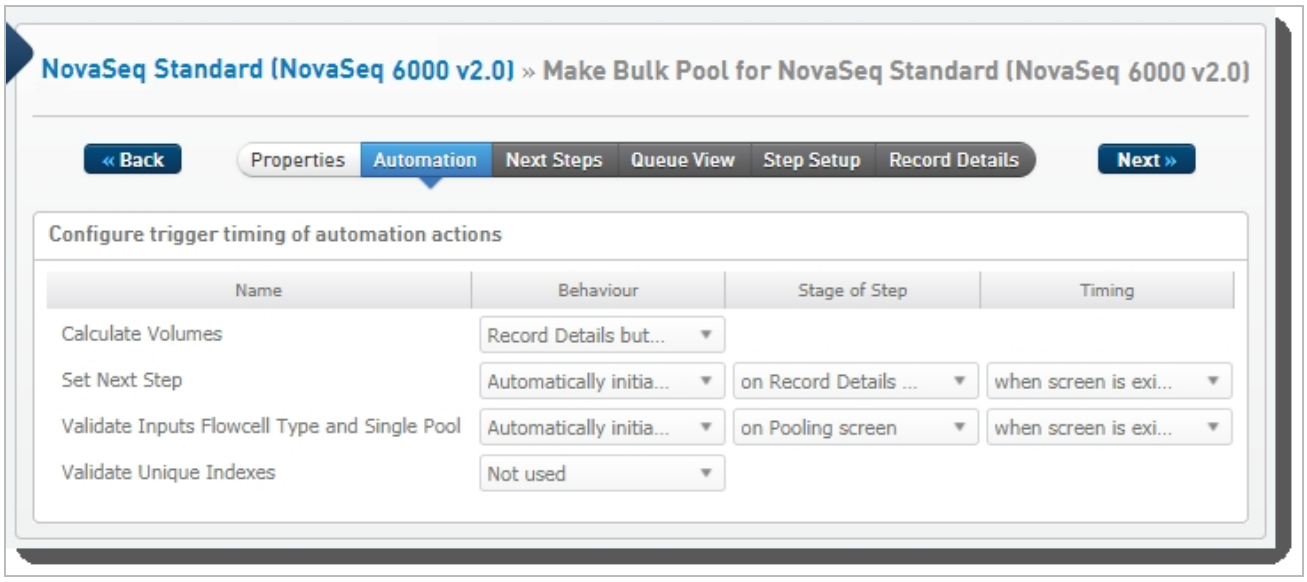
Step input: NTP (normalized libraries)

Step output: Bulk pool

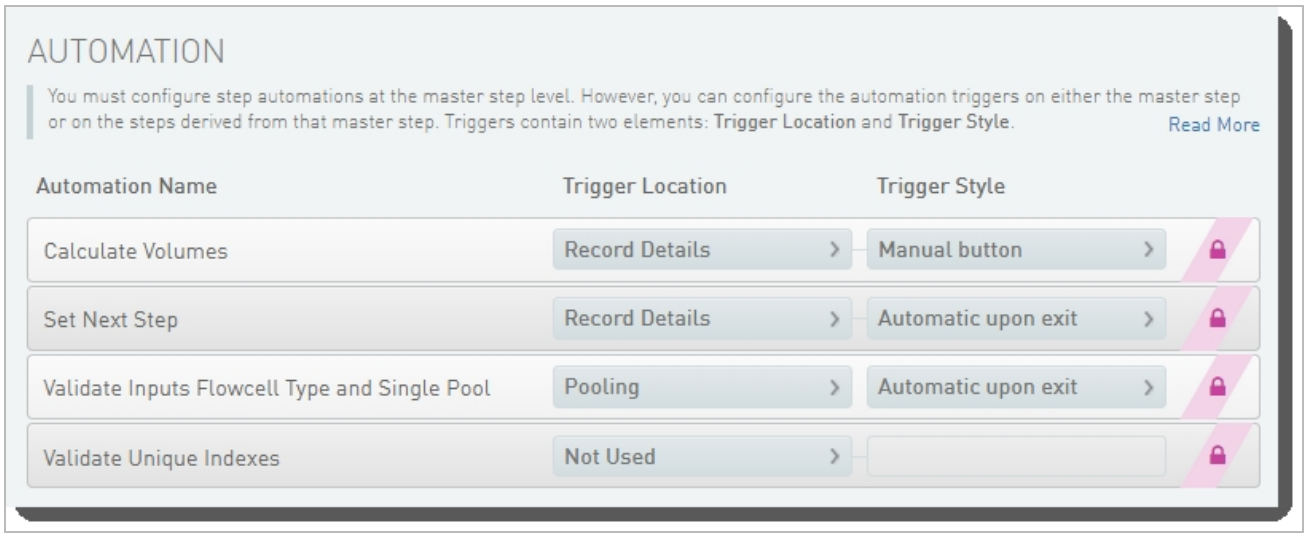
The following automations are configured on the step:

- Calculate Volumes
- Set Next Step
- Validate Inputs Flowcell Type and Single Pool
- Validate Unique Indexes*(Not used. This functionality is handled by the core LIMS configuration for pooling.)*

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Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0) automation configuration - LIMS v4.2



Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0) automation configuration - LIMS v5

These automations are described below, in the order in which they are triggered at run time.

Calculate Volumes

Automatically triggered when the user clicks the Calculate Volumes button on the Record Details screen, this automation:

- Calculates the number of samples in the pool:

```
step.::Number of Samples in Pool:: = step.::Number of Samples in Pool:: + 1
```

- Sets the value of the Bulk Pool Volume (ul) field, based on the selected Flowcell Type:

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```
if (input.::Flowcell Type:: == ::S1::) { step.::Bulk Pool Volume (ul):: =
step.::Number of Flowcells to Sequence:: * 85 + 30 }; if (input.::Flowcell Type::
== ::S2::) { step.::Bulk Pool Volume (ul):: = step.::Number of Flowcells to
Sequence:: * 150 + 30 }; if (input.::Flowcell Type:: == ::S4::) { step.::Bulk
Pool Volume (ul):: = step.::Number of Flowcells to Sequence:: * 310 + 30 };
```

- Calculates the Per Sample Volume (ul) to be added to the pool:

```
input.::Per Sample Volume (ul):: = ( ( (input.::Final Loading Concentration
(pM):: * 5 / 1000) / input.::Normalized Molarity (nM):: ) * step.::Bulk Pool
Volume (ul):: ) / step.::Number of Samples in Pool::
```

Note:
To ensure accurate pipetting of each sample in a pool for sequencing, the Per Sample Volume (ul) value must be equal to or higher than the Minimum Per Sample Volume (ul) (default value set at 5, may be edited by the user).
Assuming the default Minimum Per Sample Volume (ul) value of 5, for a given batch:
1. If the smallest Per Sample Volume (ul) value is less than 5, the LIMS automatically assigns a value of 5 to the sample's Adjusted Per Sample Volume (ul) field.
2. The LIMS then adjusts the Adjusted Per Sample Volume (ul) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

- Calculates the Total Sample Volume (ul) field value:

```
step.::Total Sample Volume (ul):: = step.::Total Sample Volume (ul):: +
input.::Adjusted Per Sample Volume (ul)::
```

- If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (ul) field value:

```
if (step.::Total Sample Volume (ul):: >= step.::Bulk Pool Volume (ul)::)
{output.::RSB Volume (ul):: = 0} else {output.::RSB Volume (ul):: = step.::Bulk
Pool Volume (ul):: - step.::Total Sample Volume (ul)::} ;
```

- Copies the Flowcell Type and Loading Workflow Type values from the step inputs to the step outputs:

```
output.::Flowcell Type::= input.::Flowcell Type::;output.::Loading Workflow
Type::= input.::Loading Workflow Type::;
```

- Sets the Volume of Pool to Denature (ul) value and calculates NaOH Volume (ul) and Tris-HCl Volume (ul) values, based on the Flowcell Type:

```
if ( input.::Flowcell Type:: == ::S1:: ) { output.::Volume of Pool to Denature
(ul):: = 85 ; output.::NaOH Volume (ul):: = 21.50 ; output.::Tris-HCl Volume
(ul):: = 21.50 } ; if ( input.::Flowcell Type:: == ::S2:: ) {output.::Volume of
Pool to Denature (ul):: = 150 ; output.::NaOH Volume (ul):: = 37.50 ;
output.::Tris-HCl Volume (ul):: = 37.50 } ; if ( input.::Flowcell Type:: ==
::S4:: ) { output.::Volume of Pool to Denature (ul):: = 310 ; output.::NaOH
Volume (ul):: = 77.50 ; output.::Tris-HCl Volume (ul):: = 77.50 }
```


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- Uses the NovaSeq_Standard_Bulk_Pool1.csv, NovaSeq_Standard_Bulk_Pool2.csv and NovaSeq_Standard_Bulk_Pool3.csv template files to generate a single CSV file containing information about the pool and the samples it contains. The generated file is available for download by the user on the Step Setup screen of the following step - Dilute and Denature (NovaSeq 6000 v2.0).

```
script:driver_file_generator \ -t
/opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Standard_Bulk_Pool1.csv \ -
o 1.csv \ script:driver_file_generator \ -t
/opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Standard_Bulk_Pool2.csv \ -
o 2.csv \ script:driver_file_generator \ -t
/opt/gls/clarity/extensions/novaseq/templates/NovaSeq_Standard_Bulk_Pool3.csv \ -
o 3.csv \&& cat 1.csv 2.csv 3.csv > {compoundOutputFileLuid0}.csv
```

Validate Inputs Flowcell Type and Single Pool

Automatically triggered on exit of the Pooling screen, this automation checks that:

- All samples in the pool have the same Flowcell Type assigned to them.
- Only one pool has been created.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/novaseq/novaseq-
extensions.jar script:validate_flowcell_for_input_pools -i {stepURI:v2} -u
{username} -p {password} -l {compoundOutputFileLuid1} -validateSingleOutput true
-poolType bulk
```

Set Next Step

Automatically triggered on exit of the Record Details screen, this automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol - Dilute and Denature (NovaSeq 6000 v2.0):

```
nextStep = ::ADVANCE::
```

Step UDFs/custom fields

There are five fields defined on the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0) step.

These are shown below in the BaseSpace Clarity LIMS v5 Configuration area (Custom Fields > Master Step Fields tab). See Table 5 for LIMS v4.2 and LIMS v5 field configuration details.

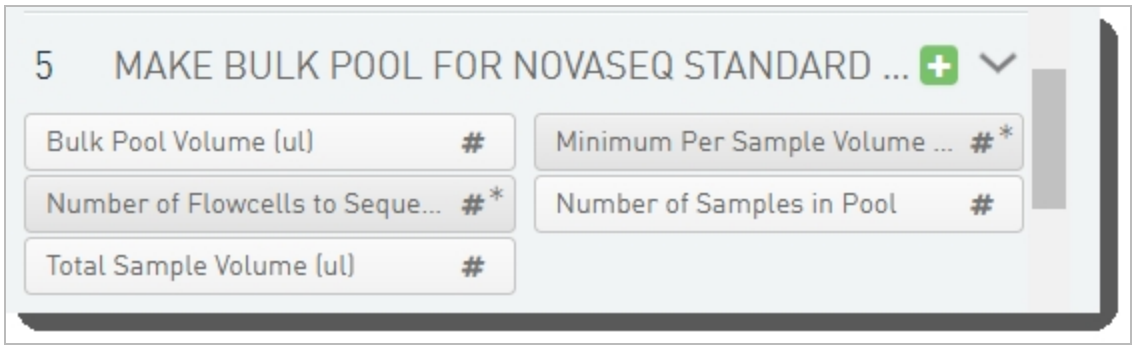


Table 5: Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0) step UDF / custom field configuration

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Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
Bulk Pool Volume (ul) (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2
Number of Flowcells to Sequence	Numeric	• Required: Yes • Read Only: No	• Range From:1 to 10 • Decimal Places Displayed: 0
Total Sample Volume (ul) (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Default: 0 • Decimal Places Displayed: 2
Minimum Per Sample Volume (uL)	Numeric	• Required: Yes • Read Only: No	• Default: 5 • Decimal Places Displayed: 2
Number of Samples in Pool (for calculation purposes, not displayed)	Numeric	• Required: No • Read Only: No	• Default: 0 • Decimal Places Displayed: 0

Global UDFs/custom fields

Table 6 lists the global UDFs / custom fields that are configured in the LIMS to support the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0) step.

Table 6: Global UDF / custom field configuration

Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
Configured on Analyte in LIMS v4.2 and Derived Samplein LIMS v5:			
Flowcell Type	Single-line Text (v4.2) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• S1 • S2 • S4

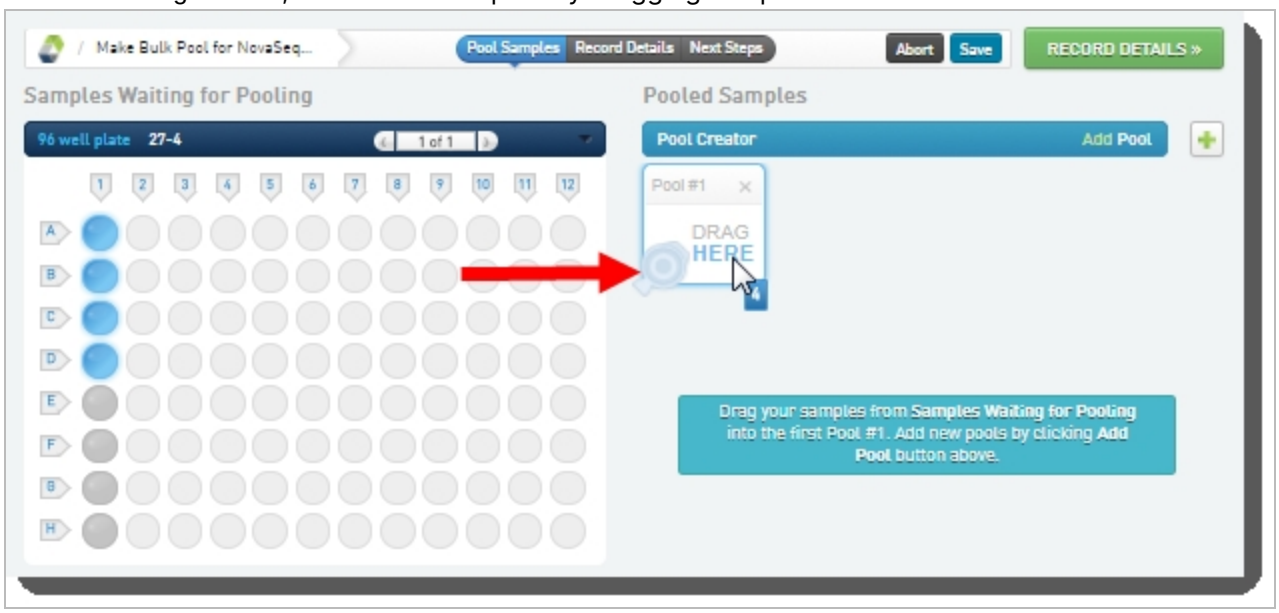
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Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
Loading Workflow Type	Single-line Text (v4.2) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • [Remove from workflow]
NaOH Volume (ul)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
RSB Volume (ul)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Tris-HCl Volume (ul)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2
Volume of Pool to Denature (ul) *Used in Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0) step only. Displays on Record Detailsscreen and in the generated CSV file.*	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0

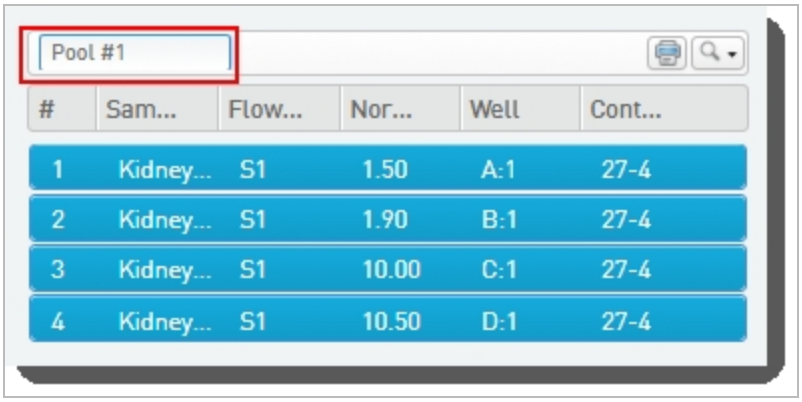
User interaction

1. On the Queue screen, user adds samples of the same Flowcell Type to the Ice Bucket and clicks View Ice Bucket.
2. On the Ice Bucket screen, user clicks Begin Work.

3. On the Pooling screen, user: Creates a pool by dragging samples into the Pool Creator.



- Names the pool, or accepts the default name - Pool #1.

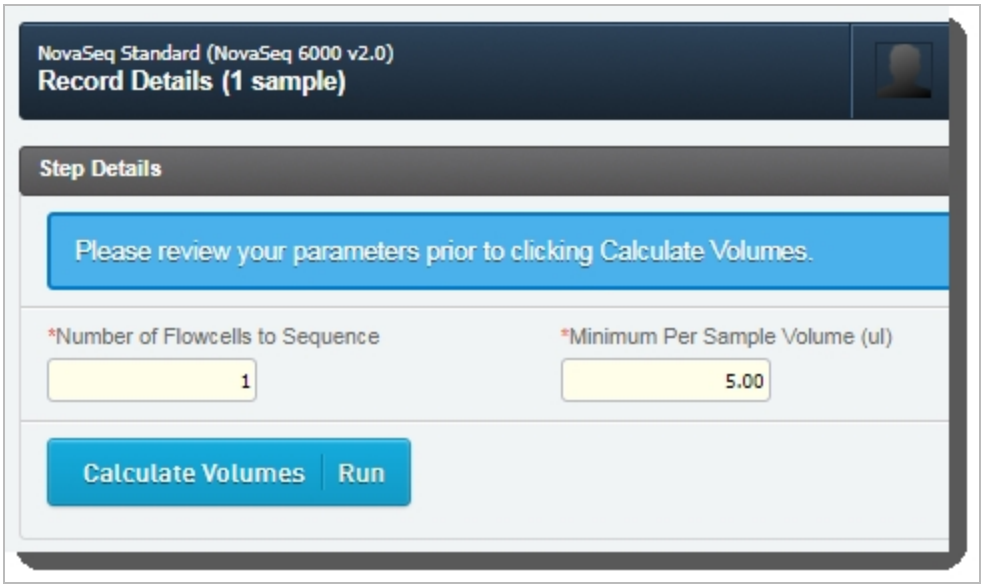


- Clicks Record DetailsOn exit from the Pooling screen, the Validate Inputs Flowcell Type and Single Pool is triggered. The automation checks that all samples in the pool have been assigned the same Flowcell Type, and only one pool has been created.

4. On the Record Details screen, the Step Details area contains two required fields:Number of Flowcells to Sequence: The value entered in this field is used in volume calculations, to ensure that volumes are sufficient for the number of times the pool will be sequenced.
    - Minimum Per Sample Volume (ul): In many applications, and particularly where robotics are used, a minimum sample volume is required. The value in this field is used to calculate how

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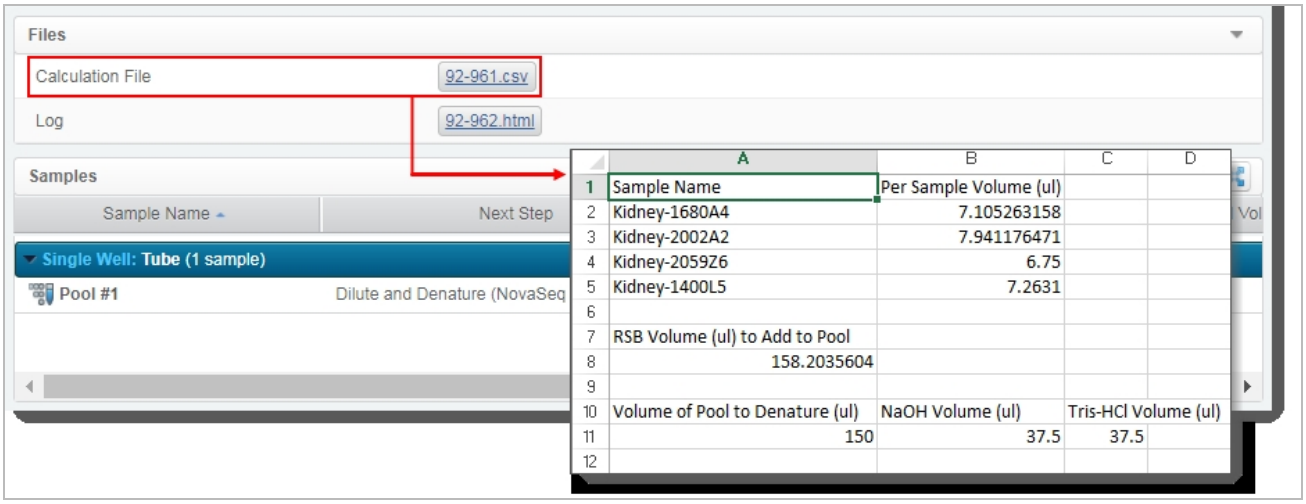
much of each sample will be included in the pool. The field is pre-populated with the configured default value - 5ul, but may be edited if required.



- In the Sample Details table, the user can click on the pool icon to view details on the pool composition.
- The user clicks Calculate Volumes. This triggers the Calculate Volumes automation, which performs volume calculations based on the selected Flowcell Type : Calculates Bulk Pool Volume (ul) value.

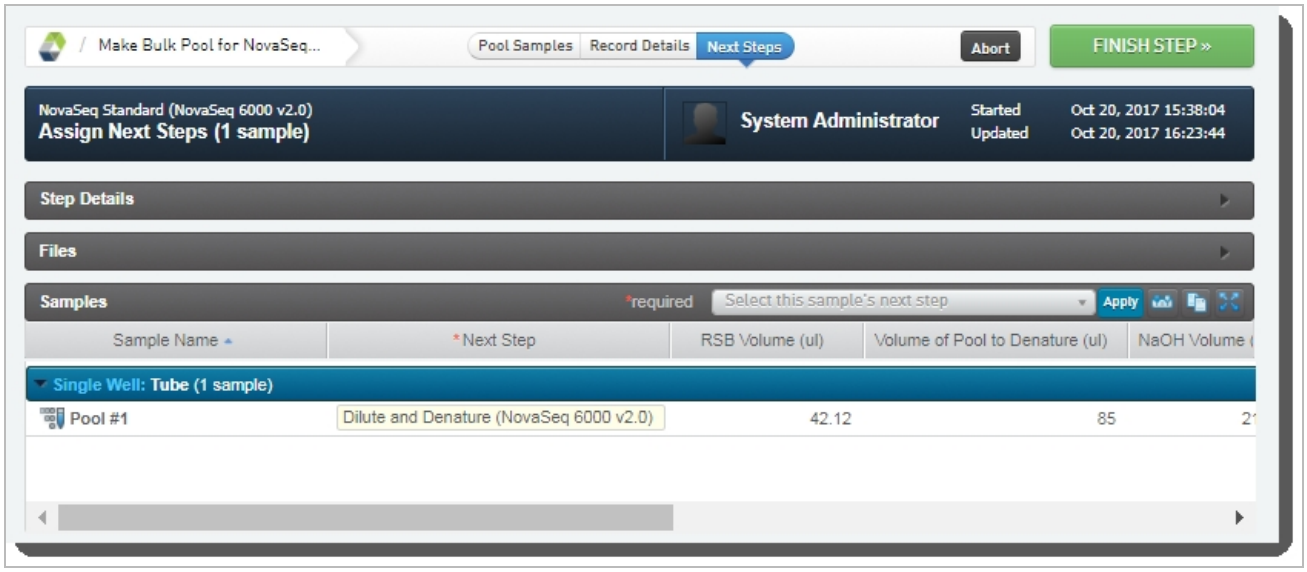
– Calculates the Per Sample Volume (ul) value to be added to the pool.
– Calculates the Total Sample Volume (ul) value.
– If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (ul) value.
– Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
– Populates the Volume of Pool to Denature (ul), NaOH Volume (ul) and Tris-HCl Volume (ul) columns of the Sample Details table. (Values are set by a script and are not editable by the user.)

– Generates the Calculation File (CSV) and attaches it to the step. This file contains volume information about the pool and the individual samples it contains. The user can click on the file to download it, and then open it in Excel.*(Click image to view full size.)*



- The user clicks Next Steps. This triggers the Set Next Step automation. This automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol - Dilute and Denature (NovaSeq 6000 v2.0).

5. On the Assign Next Steps screen: The next step is already set to Dilute and Denature (NovaSeq 6000 v2.0).



- User clicks Finish Step. At the end of this step, the pool of samples automatically advances to the Dilute and Denature (NovaSeq 6000 v2.0) step.

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Step 2: Dilute and Denature (NovaSeq 6000 v2.0)

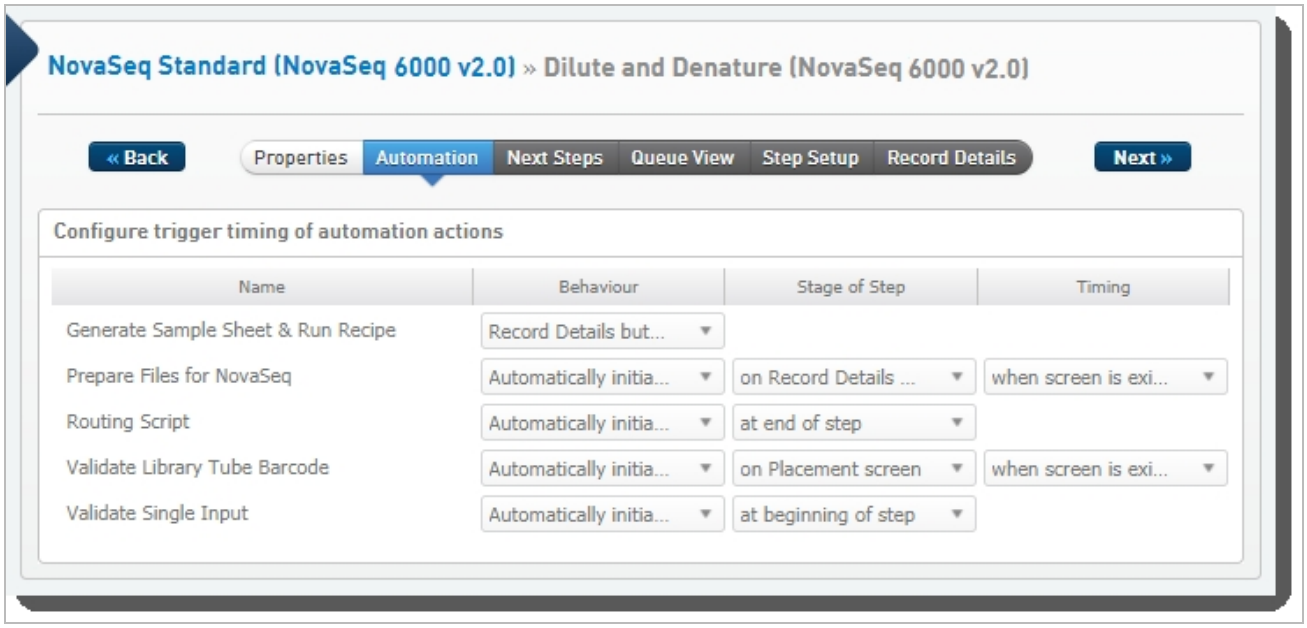
In this step, pooled samples are denatured and diluted by the addition of NaOH, Tris-HCl, and Resuspension Buffer (RSB), and are manually placed by the user into the library tube that will be used in the NovaSeq run. In addition, this step generates the sample sheet file and the run recipe (JSON file) needed to start the NovaSeq run.

Step input: Bulk pool

Step output: Library tube

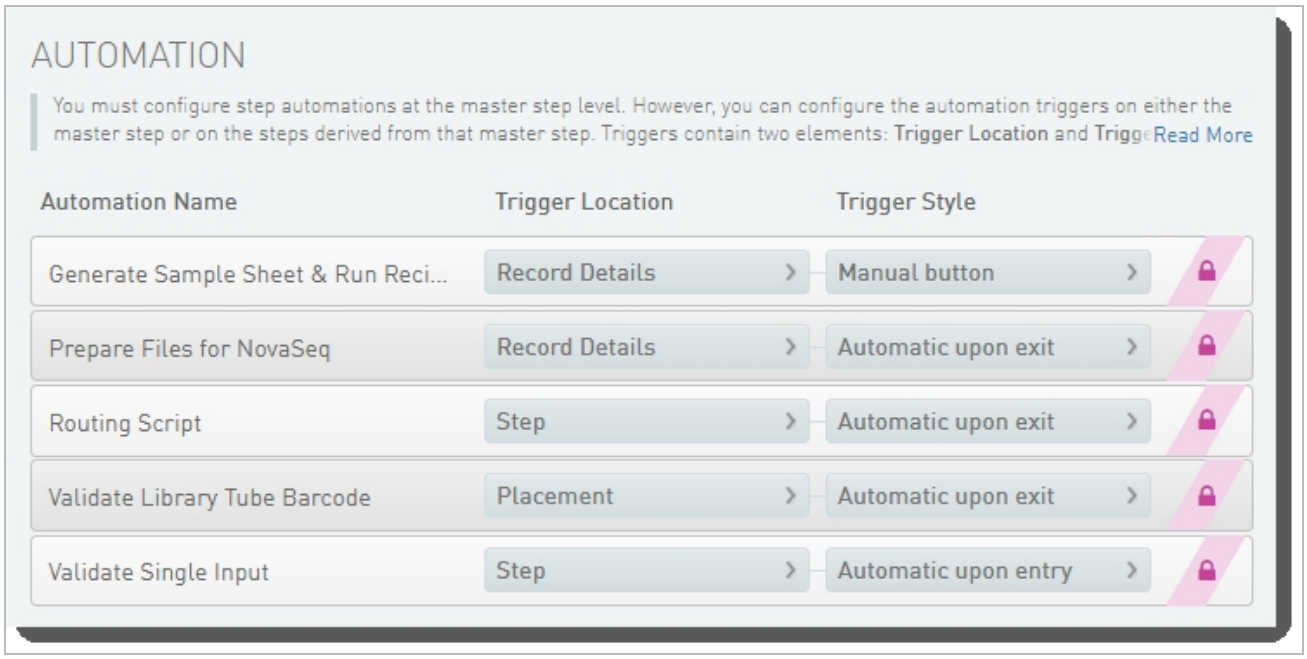
The following automations are configured on the step:

- Generate Sample Sheet and Run Recipe
- Prepare Files for NovaSeq
- Routing Script
- Validate Library Tube Barcode
- Validate Single Input



Dilute and Denature (NovaSeq 6000 v2.0) automation configuration - LIMS v4.2

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Dilute and Denature (NovaSeq 6000 v2.0) automation configuration - LIMS v5

These automations are described below, in the order in which they are triggered at run time.

Validate Single Input

Automatically triggered at the beginning of the step, this automation checks that there is only one container input to the step.

```
script:validateSampleCount -min 1 -max 1
```

Validate Library Tube Barcode

Automatically triggered on exit of the Placement screen, this automation validates the library tube barcode to ensure it conforms to the barcode mask [A-Za-z]{2}[0-9]{7}-[A-Za-z]{3}:

```
if (!output.container.name.matches(::[A-Za-z]{2}[0-9]{7}-[A-Za-z]{3}::)) { fail
(::Invalid Library Tube Barcode. Please verify and try again.::) }
```

Generate Sample Sheet and Run Recipe

Automatically triggered when user clicks a button on the Record Details screen, this automation:

- Copies the Flowcell Type from the step input to the step output:

```
output.::Flowcell Type:: = input.::Flowcell Type::
```

- Copies the Flowcell Type from the step input to the Run Mode field (hidden):

```
step.::Run Mode:: = input.::Flowcell Type::
```

- Copies the Loading Workflow Type values from the step inputs to the step outputs:

```
output.::Loading Workflow Type:: = input.::Loading Workflow Type::;
```

- Validates the parameters entered on the Record Details screen:

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  - Experiment Name may only contain alphanumeric, dash, or underscore characters. Spaces are not permitted.
  - When Workflow Type = No Index, Index Read 1 must be zero. For any other Workflow Type, Index Read 1 must be greater than zero.

```
if(!step.::Experiment Name::.matches(::[a-zA-Z0-9-_]+::)) { fail(::Experiment Name contains prohibited characters. Allowed characters are: a-z, A-Z, 0-9, -, and _::)};if(step.::Workflow Type::== ::No Index::) { if(step.::Index Read 1::!= 0) { fail(::Index Read 1 must be 0 if the Workflow Type is No Index.::) }} else { if(step.::Index Read 1::== 0) { fail(::Index Read 1 must be greater than 0 if the Workflow Type is ::+ step.::Workflow Type::+ ::.::) } };
```

- Sets Read 2 Cycles value:
  - If Paired End = True, copies the value of Read 1 Cycles into Read 2 Cycles.
  - If Paired End = False, sets Read 2 Cycles to 0.

```
step.::Read 2 Cycles:: = step.::Paired End::.toBoolean() ? step.::Read 1 Cycles:: : 0;
```

- Determines Index Read 2 based on the Workflow Type and Index Read 1: When Workflow Type = No Index or Single Index, Index Read 2 is set to 0.
  - When Workflow Type = Dual Index or Custom, Index Read 2 is set to the same as Index Read 1.

```
if (step.::Workflow Type:: == ::No Index:: || step.::Workflow Type:: == ::Single Index::) { step.::Index Read 2:: = 0 } else { step.::Index Read 2:: = step.::Index Read 1:: }
```

- Sets the next step for samples to REMOVE:

```
nextStep = ::REMOVE::
```

- Generates the sample sheet and the run recipe file and attaches them to the step. For details on how the files are generated, and their contents, see Sample sheet and run recipe file generation section, below.

Prepare Files for NovaSeq

Automatically triggered on exit of the Record Details screen, this automation invokes the copy_attachments_to_network_folder script, which is included in the novaseq-remote-extensions.jar file. This script places the generated sample sheet and run recipe (JSON file) into the appropriate folder on the NAS, where the instrument will use them to start the run.

For more information, see Sample sheet and run recipe file generation section, below.

Routing Script

Automatically triggered on exit of the step, this automation invokes the changeWorkflow script, which routes step outputs to the NovaSeq 6000 v2.0 workflow, and queues them for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) step.

Default automation command line:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -u {username} -p {password} -i {stepURI:v2} -l
{compoundOutputFileLuid2} script:changeWorkflow \ \ --FIELD_NAME 'N/A' \ --FIELD_
VALUE 'N/A' \ --WORKFLOW 'NovaSeq 6000 v2.0' \ --STEP 'AUTOMATED - NovaSeq Run
(NovaSeq 6000 v2.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS'"
```

Step UDFs/custom fields

There are 14 fields defined on the Dilute and Denature (NovaSeq 6000 v2.0) step. These fields are required for sample sheet and JSON file generation..

These fields are shown below in the BaseSpace Clarity LIMS v5 Configuration area (Custom Fields > Master Step Fields tab). See Table 7 for LIMS v4.2 and LIMS v5 field configuration details.

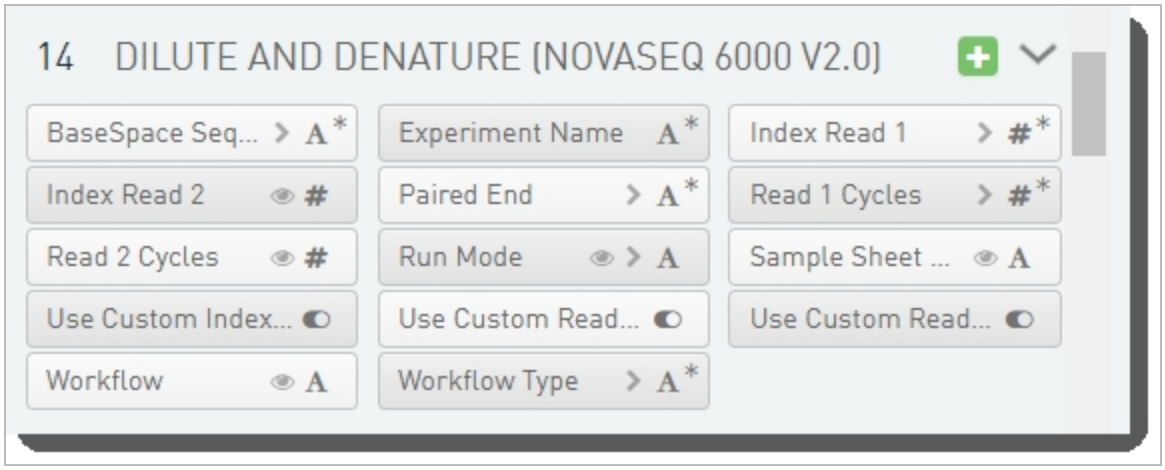


Table 7: Dilute and Denature (NovaSeq 6000 v2.0) step UDF / custom field configuration

Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
BaseSpace Sequence Hub Configuration	Single-line Text (v4.2) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• Not Used • Run Monitoring Only • Run Monitoring and Storage
Experiment Name	Single-line Text (v4.2) Text (v5)	• Required: Yes • Read Only: No	
Index Read 1	Numeric (v4.2) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 0 • 6 • 8 • Range From: 0 to 20 • Decimal Places Displayed: 0

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Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
Index Read 2	Numeric (v4.2) Numeric (v5)	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0
Paired End	Single-line Text (v4.2) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• True • False
Read 1 Cycles	Numeric (v4.2) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 151 • 101 • 51 • Range From: 1 to 151 • Decimal Places Displayed: 0
Read 2 Cycles	Numeric (v4.2) Numeric (v5)	• Required: No • Read Only: Yes	• Decimal Places Displayed: 0
Run Mode *(not displayed in user interface)*	Single-line Text (v4.2) Text Dropdown (v5)	• Required: No • Read Only: Yes • Custom Entries: No	• S1 • S2 • S4
Sample Sheet Path	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: Yes	
Use Custom Index Read 1 Primer	Check Box (v4.2) Toggle Switch (v5)	• Required: No • Read Only: No	• None Set
Use Custom Read 1 Primer	Check Box (v4.2) Toggle Switch (v5)	• Required: No • Read Only: No	• None Set
Use Custom Read 2 Primer	Check Box (v4.2) Toggle Switch (v5)	• Required: No • Read Only: No	• None Set

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Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
Workflow	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: Yes	GenerateFASTQ
Workflow Type	Single-line Text (v4.2) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• No Index • Single Index • Dual Index • Custom

Global UDFs/custom fields

Table 8 lists the global UDFs / custom fields that are configured in the LIMS to support the Dilute and Denature (NovaSeq 6000 v2.0) step.

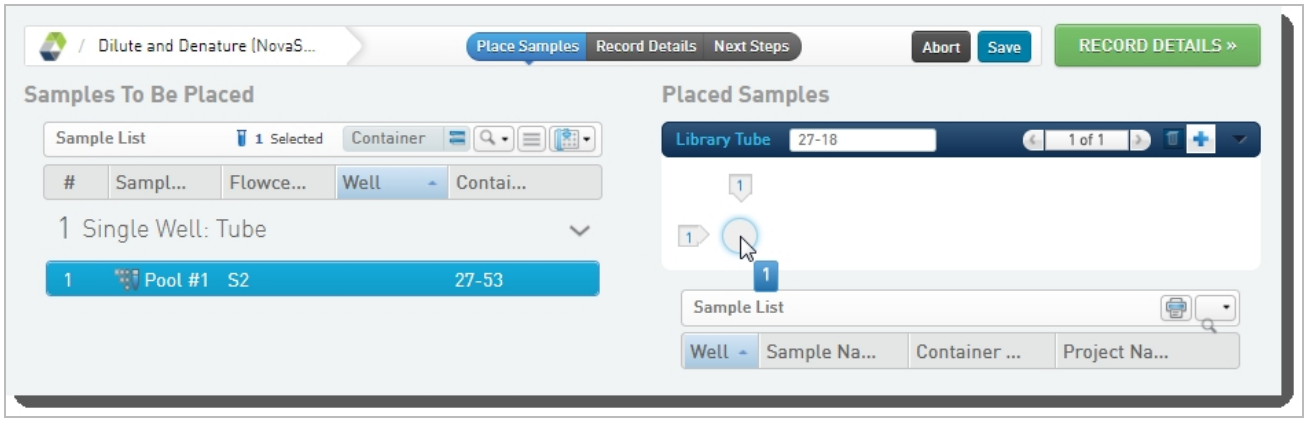
Table 8: Global UDF / custom field configuration

Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
Configured on Analyte in LIMS v4.2 and Derived Samplein LIMS v5:			
Flowcell Type	• Single-line Text (v4.2) • Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• S1 • S2 • S4
Loading Workflow Type	• Single-line Text (v4.2) • Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• NovaSeq Standard • [Remove from workflow]

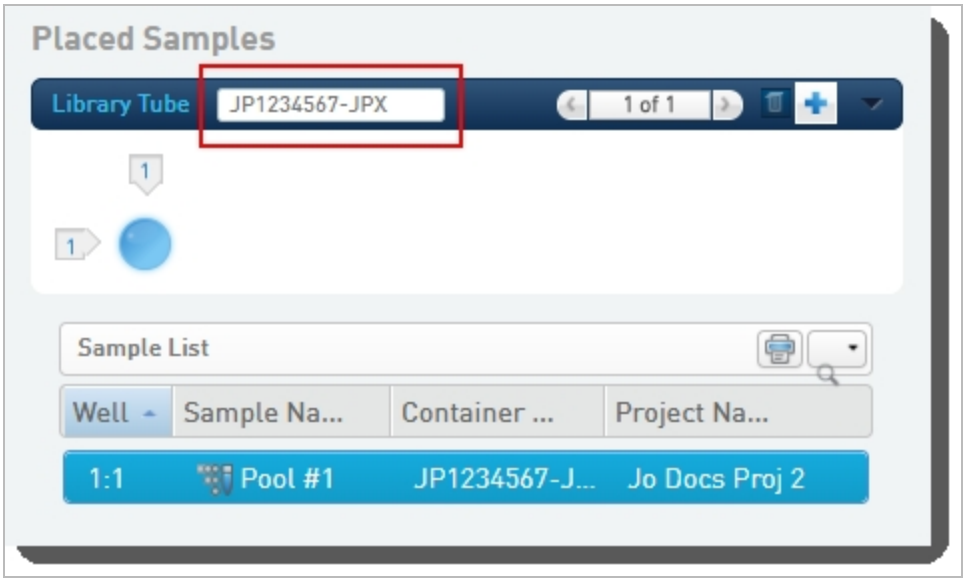
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User interaction

1. On the Placement screen, user does the following: Drags the pool into the library tube in the Placed Samples area.



   - Scans or types the barcode of the library tube into the Library Tubefield.

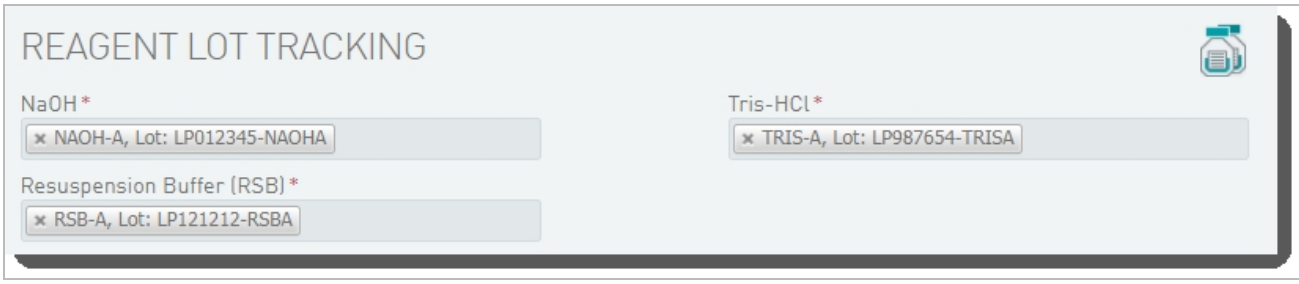


   - Clicks Record Details.On exit of the Placement screen, the Validate Library Tube Barcode automation checks that the library tube barcode conforms to the barcode mask [A-Za-z]{2}[0-9]{7}-[A-Za-z]{3}, and displays an error message if this is not the case.

2. On the Record Details screen:

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- The Reagent Lot Tracking section lets you track the NaOH, Resuspension Buffer, and Tris-HClreagents used in the step. The user selects from the active lots displayed in each drop-down list.



- The fields displayed in the Step Details section are used to generate the sample sheet and run recipe files. Some of these fields are auto-populated and some must be completed by the user (see Table 9, below, for details).

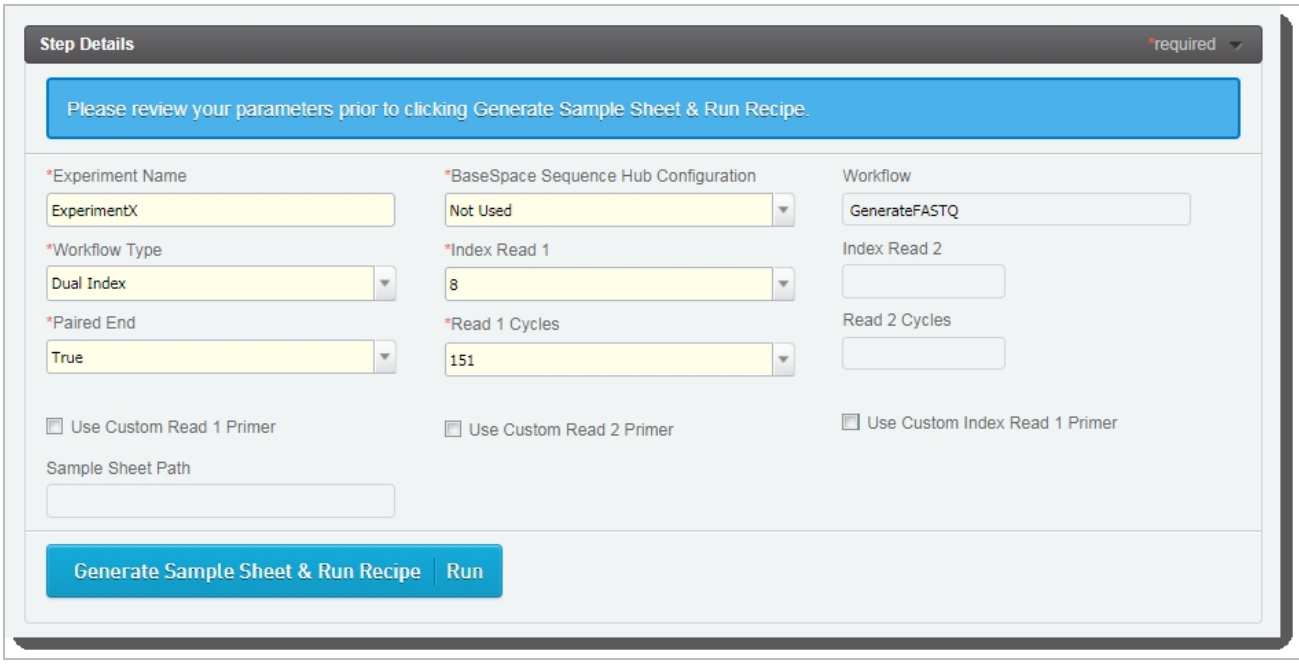


Table 9: Fields displayed on Record Details screen of Dilute and Denature (NovaSeq 6000 v2.0) step

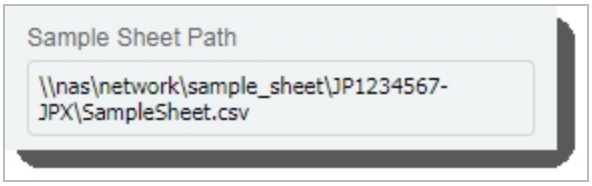
Field	Value
Experiment Name	User enters the name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.
BaseSpace Sequence Hub Configuration	User selects from preset options: Not Used, Run Monitoring Only, or Run Monitoring and Storage
Workflow Type	User selects from preset options: No Index, Single Index, Dual Index, or Custom

Field	Value
Index Read 1	User selects from preset option: 0, 6, or 8 - or types a value between 0 and 20
Index Read 2	If user selected the Dual Index workflow type, once the sample sheet has been generated, Index Read 2 value is automatically populated to match Index Read 1.
Paired End	User selects from preset options: True or False
Read 1 Cycles	User selects from preset options: 151, 101, or 51 - or types a value between 1 and 151
Read 2 Cycles	If user selected Paired End =True, once the sample sheet has been generated, Read 2 Cycles value is automatically populated to match Read 1 Cycles.
Use Custom Read 1 Primer	User selects if applicable.
Use Custom Read 2 Primer	User selects if applicable.
Use Custom Index Read 1 Primer	User selects if applicable.
Sample Sheet Path	Automatically populated once the sample sheet has been generated.
Workflow	Automatically populated with preset - GenerateFASTQ.

3. On the Record Details screen, user clicks Generate Sample Sheet and Run Recipe . This triggers the automation script, which does the following.Generates the sample sheet and JSON files and attaches them to the placeholders in the Files area of the Record Detailsscreen.



- Populates the Sample Sheet Pathfield with the path to the sample sheet file.



- If applicable, populates the Index Read 2 and Read 2 Cycles fields.
- Sets the value of the next step to Remove from workflow. The Routing Script automation expects this value, and requires it in order to successfully advance samples to the next step.
- User clicks Next Steps. This triggers the Prepare Files for NovaSeq automation, which copies the sample sheet and run recipe files to the location specified during installation.

4. On the Assign Next Steps screen:
    - The Next Step for all samples is pre-populated with Remove from workflow.

    The user must not change this value! If Next Step is not set to Remove from workflow, the routing script will not be able to route samples correctly.

    - User clicks Finish Step.
5. On exit from the step: The Routing Script automation is triggered and samples are routed to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) protocol.
    - In Lab View, the pool of samples is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) step.

At this point in the workflow, the user interaction ends. The integration automatically starts the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) step and data from the run is parsed back into the LIMS.

Protocol 3: NovaSeq Xp (NovaSeq 6000 v2.0)

In this release, the NovaSeq Xp (NovaSeq 6000 v2.0) protocol is hidden and does not display in Lab View.

This protocol allows for the support of functionality that will be used in a future release.

Do not delete or modify the configuration of the protocol, or the steps it contains.

Protocol 4: AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0)

This final protocol contains one fully-automated step, AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0), described below.

This is a fully-automated step. The user must not add samples to the Ice Bucket or start the step manually.

Step 1: AUTOMATED - NovaSeq Run (NovaSeq 6000) 1.0

Step input: Library tube from NovaSeq Standard protocol

Step output: Result file / measurement

The user must not start this step. This is a fully-automated step and the sequencing service will not update samples correctly if they have been manually started.

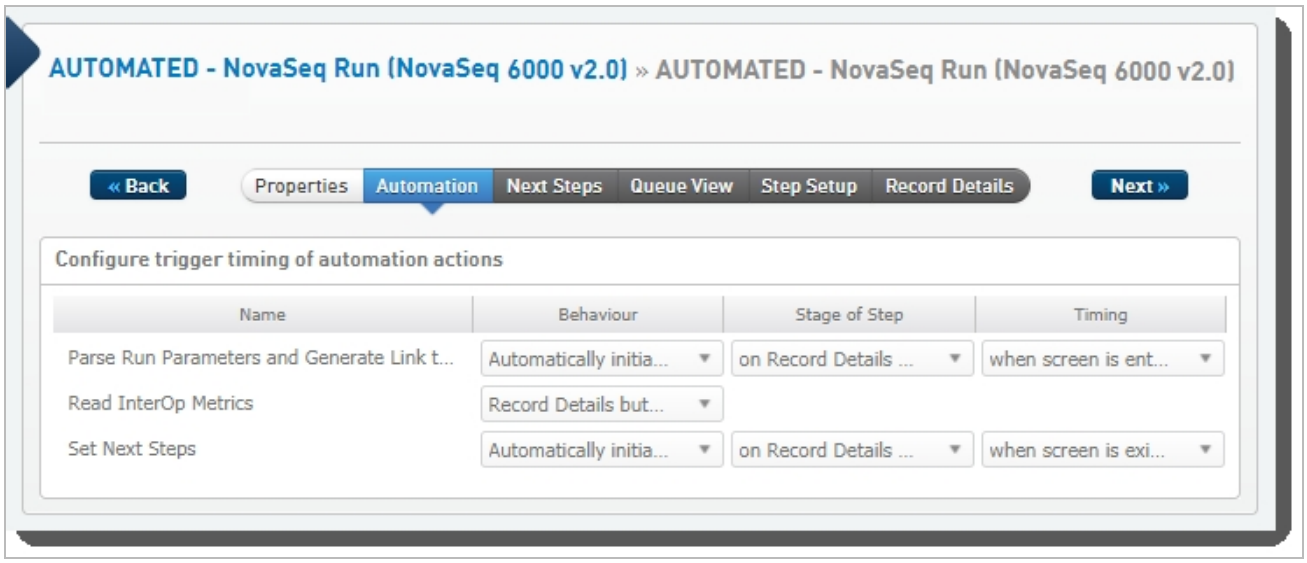
In this step, pooled samples are sequenced on the NovaSeq instrument. The run data network folder is monitored by the sequencing service and automation worker to determine when the sequencing run begins and ends.

1. When the user initiates a run on the NovaSeq instrument, the NovaSeq Control Software (NVCS) looks for the sample sheet and run recipe (*.json file) on the shared network drive.The NVCS copies the sample sheet to the run directory.
    - The NVCS uses the run recipe to start the sequencing run.
2. When the run starts: The instrument software creates a new run folder (named < libraryID >) on the shared network drive and copies the following files into that folder:RunParameters.xml
    - RunInfo.xml
    - LIMS/ <libraryID>.json file (run recipe)
        - The Real-Time Analysis v3 (RTA3) software creates the InterOp folder.
3. The sequencing service detects the presence of the RunParameters, RunInfo, and JSON files and starts the AUTOMATED- NovaSeq Run (NovaSeq 6000 v2.0) step in the LIMS.
4. As the run progresses, the InterOp data files are filled in.
5. At the end of the run, the NovaSeq: Copies the run data files to the InterOp folder.
    - Creates the CopyComplete.txt file in the run folder. This indicates to the sequencing service that the run has completed and the data files are ready.
6. The sequencing service triggers the Read InterOp Metrics automation, which reads the files, records the parsed metrics into the LIMS, and finally completes the step in LIMS.

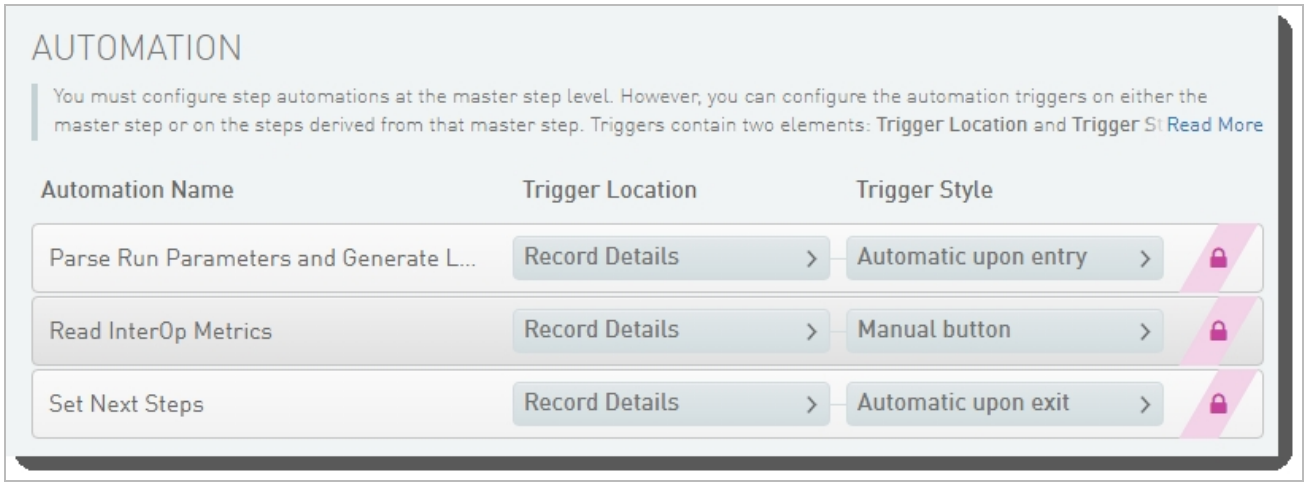
The following automations are configured on the step:

- Parse Run Parameters and Generate Link to Run Folder
- Read InterOp Metrics
- Set Next Steps

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AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) automation configuration - LIMS v4.2



AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) automation configuration - LIMS v5

These automations are described below, in the order in which they are triggered at run time.

Parse Run Parameters and Generate Link to Run Folder

Automatically triggered on entry to the Record Details screen, this automation:

- Parses the RunParameters.xml file attached on the Step Setup screen.
- Populates the step UDFs / custom fields with extracted values.
- If there is an instrument in the LIMS that matches the instrument name in the file (InstrumentName field), the script associates this instrument with the run.
- Generates and attaches a link to the run folder using the run output folder parsed from RunParameters.xml.

Rules and constraints

- If there is no InstrumentName value in the file, the script continues, but logs a warning.
- If there is an InstrumentName value in the file and multiple instruments with the same name exist in the LIMS, the script fails and logs an error.
- If the RunParameters.xml file is not attached to the step during step setup, the script fails.
- Every RunParameters.xml field that has an equivalent step UDF / custom field in the LIMS is required - except for InstrumentName. If a field is not present in the RunParameters.xml file, the LIMS logs an error to this effect.

Step UDFs/custom fields

Table 10, below, shows how the parsed RunParameters.xml fields map to step UDFs / custom fields configured on the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) step in the LIMS.

Table 10: Fields parsed by parse_novaseq_run_parameters script

RunParameters.xml Data	*LIMS Step UDF / Custom Field Configuration*
RunParameters field	Step UDF / Custom Field Name
FlowCellSerialBarcode	Flow Cell ID
FlowCellPartNumber	Flow Cell Part Number
FlowCellLotNumber	Flow Cell Lot Number
FlowCellExpirationdate	Flow Cell Expiration Date
FlowCellMode	Flow Cell Mode
RunId	Run ID
ClusterSerialBarcode	PE Serial Barcode
ClusterPartNumber	PE Part Number
ClusterLotNumber	PE Lot Number
ClusterExpirationdate	PE Expiration Date
ClusterCycleKit	PE Cycle Kit
SbsSerialBarcode	SBS Serial Barcode
SbsPartNumber	SBS Part Number
SbsLotNumber	SBS Lot Number
SbsExpirationdate	SBS Expiration Date
SbsCycleKit	SBS Cycle Kit
BufferSerialBarcode	Buffer Serial Barcode
BufferPartNumber	Buffer Part Number
BufferLotNumber	Buffer Lot Number
BufferExpirationdate	Buffer Expiration

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RunParameters.xml Data	*LIMS Step UDF / Custom Field Configuration*
OutputRunFolder	Output Folder
WorkflowType	Loading Workflow Type

Read InterOp Metrics

Automatically triggered when the sequencing service detects the CopyComplete.txt file has been created, this automation parses the InterOp data generated by the run.

- Read summary metrics are recorded on the library pool. These values are aggregated across all lanes. Some values - for example Yield PF (Gb) - are summed, while others are averaged.
- Per-lane metrics are recorded on the measurement outputs.

The read_interop_metrics script loads data from the InterOp files and records summary metrics into derived sample UDFs / custom fields in the LIMS. The script will fail if any field is not configured or cannot be written to.

The following key metrics are parsed back into the LIMS:

- Yield PF (Gb) R1
- Yield PF (Gb) R2
- Reads PF (M) R1
- Reads PF (M) R2
- Cluster Density (K/mm^2) R1
- Cluster Density (K/mm^2) R2
- %PF R1
- %PF R2
- % Bases >=Q30 R1
- % Bases >=Q30 R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- % Aligned R1
- % Aligned R
- % Error Rate R1
- % Error Rate R2

Set Next Steps

Automatically triggered on exit of the Record Details screen, this automation sets the next step to ADVANCE and the samples complete the protocol.

```
nextStep = ::ADVANCE::
```

Components installed with this integration

The following sections describe the various components - files, database properties, reagent categories / label groups, reagent kits, and containers - that are installed by default as part of this integration. Information on installed workflows, protocols, steps, and automation points is provided in the Workflows, protocols and steps section, above.

The Illumina NovaSeq 6000 Integration v2.1 is distributed as two RPM packages:

- BaseSpaceLIMS-novaseq-extensions
- BaseSpaceLIMS-novaseq-sequencing-service

The RPM packages install the components listed in the following table.

Note that the NovaSeq 6000 Integration requires installation of NGS Extensions Package v5.15 or later.

- If the NGS Extensions Package is not already installed, or if a version earlier than than v5.15 is installed, v5.16 is installed by default with the NovaSeq integration. See Release Notes for details.
- If NGS Extensions Package v5.15 is already installed, upgrade is not forced or required.

Table 11: Illumina NovaSeq 6000 Integration RPM components

Files Installed	Location and Description
configure_extensions_novaseq.sh	*/opt/gls/clarity/config/* Script that imports LIMS configuration into the application database and updates property values.
novaseq-extensions.jar	*/opt/gls/clarity/extensions/novaseq* Jar file containing API-based LIMS extensions used throughout the protocols.
novaseq-sequencing.jar	*/opt/gls/clarity/extensions/novaseq/SequencingService* Sequencing service jar file that captures the run results.
novaseq-remote-extensions.jar	*/opt/gls/clarity/extensions/novaseq* Jar file containing scripts that require access to the run folders on the NAS.
InterOp libraries	*/opt/gls/clarity/extensions/novaseq/lib* Illumina shared library for parsing InterOp data files.

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Files Installed	Location and Description
• NovaSeq_Standard_Bulk_Pool1.csv • NovaSeq_Standard_Bulk_Pool2.csv • NovaSeq_Standard_Bulk_Pool3.csv • NovaSeq_Xp_Bulk_Pool.csv • NovaSeq_Xp_Bulk_Pool2.csv • NovaSeq_Xp_Working_Pool.csv • NovaSeq_Xp_Working_Pool2.csv	*/opt/gls/clarity/extensions/novaseq/Templates* Template files used for file generation throughout the protocols.

Database properties

Table 12 lists the database properties installed with the Illumina NovaSeq 6000 Integration Package.

Table 12: Database properties installed

Property	Description
novaseq.runSetupFolder	The directory where the run recipe files will be written.
novaseq.sampleSheetPathPrefixSearch.SUFFIX	The directory where the instrument control software or analysis software looks for sample sheets, typically a Windows path to a network drive.
novaseq.sampleSheetPathPrefixReplace.SUFFIX	The directory where the sample sheets will be written.
novaseq.sampleSheetPathPrefixSearchReplaceSuffixes*	Search and replace entries for transforming Windows to Linux network paths. This property must be set to the single SUFFIX used in the two properties above.
novaseq.seqservice.netPathPrefixSearch.SUFFIX	The search prefix used to map the OutputRunFolder field in RunParameters.xml to the corresponding Linux directory.
novaseq.seqservice.netPathPrefixReplace.SUFFIX	The directory where the sequencing service will look for run folders.

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Property	Description
novaseq.seqservice.netPathPrefixSearchReplaceSuffixes*	Search and replace entries for transforming Windows to Linux network paths. This property must be set to the single SUFFIX used in the two properties above.

**This version of the integration only supports a single active search-replace pair of each type: sampleSheetPathPrefixSearchReplaceSuffixes and netPathPrefixSearchReplaceSuffixesmust each contain a single number, rather than a comma-separated list of numbers.*

Reagent categories/label groups

- TruSeq HT Adapters v2 (D7-D5)

Reagent kits

- DPX1
- DPX2
- DPX3
- Resuspension Buffer (RSB)
- NaOH
- Tris HCl

Container types

- Library Tube

Instrument integration

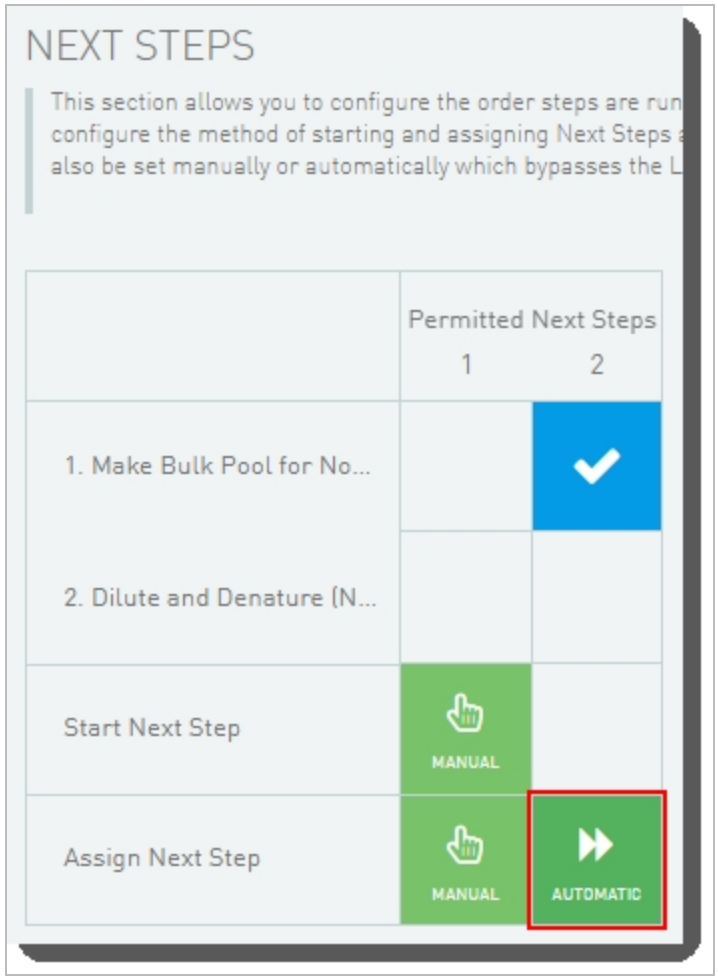
Instructions for configuring the NovaSeq instrument for LIMS integration are provided in the *NovaSeq Sequencing System Guide*.

Routing script requirements

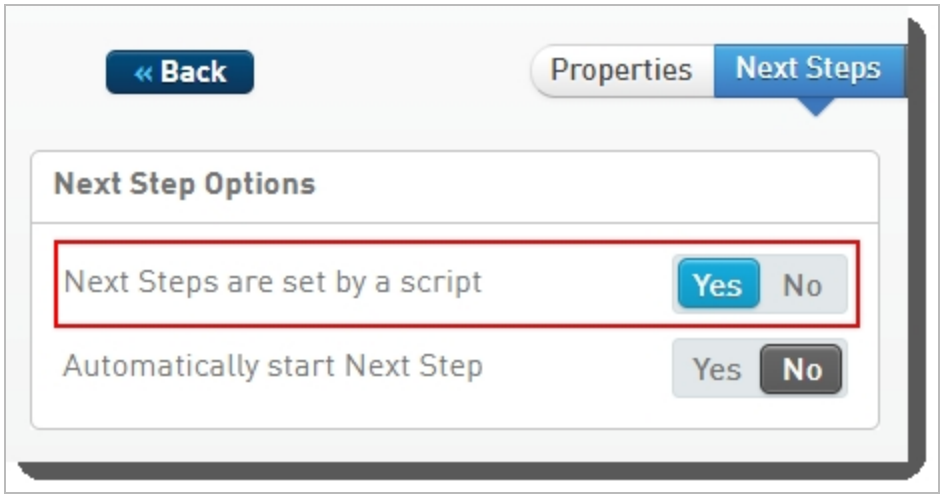
The requirements for the routing script functionality are as follows:

- On the steps that use the routing script - Define Run Format (NovaSeq 6000 v2.0) and Dilute and Denature (NovaSeq 6000 v2.0) - the Next Step for all samples must be set to Remove from workflow. This value is set by a script, and the user running the step must not change this value in the Assign Next Steps screen.

- LIMS v5: In the protocol configuration screen, the following settings are required and must not be changed:In the Next Steps section, for the last step in the protocol, the method of assigning the next step must be set to Automatic.



- LIMS v4.2: In the protocol configuration area, for all protocol steps on which the Routing Script automation is configured, the following settings are required and must not be changed:
  On the Next Steps configuration tab, the Next Steps are set by a script toggle switch must be set to Yes.



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Sample sheet and run recipe file generation

Sample sheet and run recipe file generation occurs on the step prior to the run – Dilute and Denature (NovaSeq 6000 v2.0) – which is the step where samples are placed on the library tube that will be loaded in the instrument.

In the out-of-the-box configuration, the Generate Sample Sheet and Run Recipe automation generates the following:

- One CSV format sample sheet file for use bcl2fastq v2.19 downstream analysis
- One JSON format run recipe file

The Prepare Files for NovaSeq automation, which runs at the end of the step, places the generated files on the NAS where the instrument will use them to start the run.

How it works

- The Prepare Files for NovaSeq automation command line invokes the copy_attachments_to_network_folder script in novaseq-remote-extensions.jar .The script may be used on a remote AI node / automation worker, in case the LIMS server does not have direct access to the NAS.
- The output folder for the run recipe file is found in the novaseq.runSetupFolder property.
- The output folder for the sample sheet is determined by using the novaseq.sampleSheetPathSearchReplaceSuffixes property. This property must contain exactly one suffix (not a comma-separated list) to derive a full property name based on novaseq.sampleSheetPathPrefixReplace.<suffix>.

Sample sheet contents

The contents of the sample sheet are controlled by the following fields configured on the Dilute and Denature (NovaSeq 6000 v2.0) step:

- Experiment Name
- Read 1 Cycles
- Read 2 Cycles
- Workflow

See the *Bcl2fastq2 Sample Sheet Generation* article for detailed information on the following:

- Sample sheet generation script parameters and usage
- Sample sheet data and configuration options
- Enabling unique FASTQ file names per sequencing run

Run recipe contents

The contents of the run recipe are controlled by the following fields. These fields are configured on the Dilute and Denature (NovaSeq 6000 v2.0) step.

- Experiment Name
- Run Mode

- Workflow Type
- Sample Loading Type (populated based on Workflow field)
- Paired End
- Read 1
- Read 2
- Index Read 1
- Index Read 2
- Sample Sheet Path
- BaseSpace Configuration
- Use Custom Read 1 Primer
- Use Custom Read 2 Primer
- Use Custom Index Read 1 Primer

For field configuration details, see Table 7, above. To see how these fields are populated on the Record Details screen at run time, see Table 9.

The following fields are also included in the run recipe:

- Output Container Name (*librarytube_ID* in JSON file): The library tube barcode derived from the container name entered or scanned on the Placement screen
- Rehyb - preset to False
- Output Folde r (*output_folder* in JSON file): Derived from the system property novaseq.seqservice.netPathPrefixSearch
- Attachment: Derived from the system property novaseq.sampleSheetPathPrefixSearch with the name of the sample sheet added to the end of the path
- Use BaseSpace (*use_basespace* in JSON file): Set to False if the BaseSpace Sequence Hub Configuration step UDF / custom field value is 'Not Used', and true otherwise

Rules and constraints

- The sample sheet and run recipe files are mandatory inputs for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) step. For the automated run to start successfully, the user must have clicked the Generate Sample Sheet and Run Recipe button to generate these files.
- The output folders must exist on the system where the script runs (this may be a remote AI node / automation worker).
- The output folders must be writable by the glsai user.

Support for container types

This integration supports the Library Tube container type with barcode provided in the format [A-Za-z]{2}[0-9]{7}-[A-Za-z]{3} - for example, AB1234567-XYZ.

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Rules and constraints

- The workflow configuration contains several validation checks. To ensure that the calculations work properly, it is important that you do not disable any of this validation logic. The validation checks determine:Which samples, and how many, can enter each step together.
  - Which samples, and how many, can be pooled together.
- Reagent labels (indexes) must be unique.
- The library tube ID must be unique. There should not be multiple library tube containers in the system with the same name.
- Only controls are permitted as unindexed samples; all other unindexed samples and pools are not permitted.
- For sample sheet generation constraints, refer to the *Bcl2fastq2 Sample Sheet Generation* article.
- Users must not manually start the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) step. This is a fully-automated step and the sequencing service will not update samples correctly if they have been manually started.
- The output folder must be readable by the LIMS server's glsjboss account. For details, see *Installation Guide*.

Illumina NovaSeq 6000 Integration v2.1 - Validation and Troubleshooting

This guide explains how to validate the installation of the Illumina NovaSeq 6000 Integration Package v2.1.

The validation process involves:

- Running samples through the NovaSeq Validation Library Prep (NovaSeq 6000 v2.0) workflow.
  - The workflow contains a single-step protocol that models the library prep required to produce normalized libraries. At the end of the step, the normalized libraries are automatically advanced to the NovaSeq 6000 v2.0 workflow.
- Running normalized libraries through the NovaSeq 6000 v2.0 workflow. This validates the following:
  - Successful routing of samples from the Run Format (NovaSeq 6000 v2.0) step to the NovaSeq Standard (NovaSeq 6000 v2.0) step.
  - Automated generation of a sample sheet for use with bcl2fastq2 v2.19 analysis software.
  - Automated generation of a run recipe file (JSON file) with the library tube barcode as the name (e.g., AB1234567-CDE.json). This file is automatically uploaded to the sequencing instrument and used to set up and initiate the run.
  - Automated tracking of the NovaSeq sequencing run and parsing of run statistics (per run per lane) into the LIMS.

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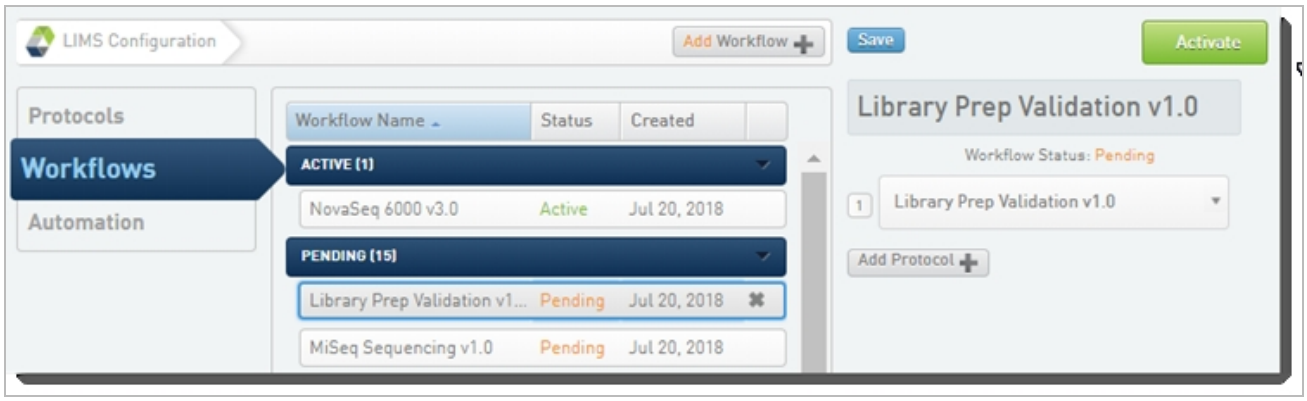
Before you begin

Before you begin to execute the validation steps below, note that these steps assume that you have installed the Illumina NovaSeq 6000 Integration Package v2.1 and have imported the default LIMS configuration.

Validation steps

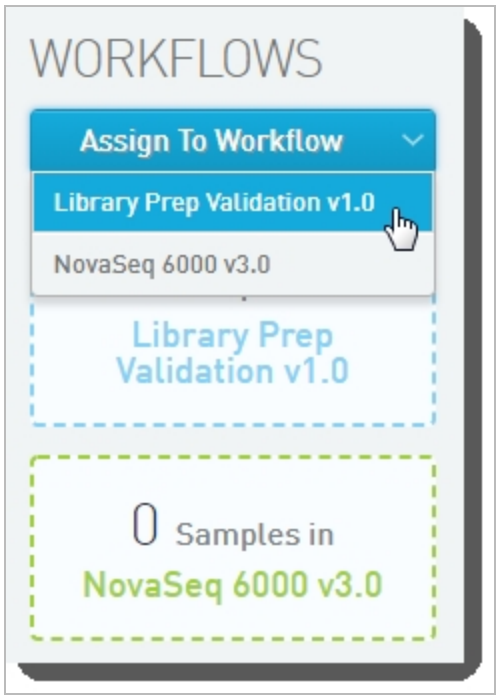
1. Activate the workflow

- In the LIMS configuration area, activate the NovaSeq Validation Library Prep (NovaSeq 6000 v2.0) and NovaSeq 6000 v2.0 workflows.



2. Create a project, add samples

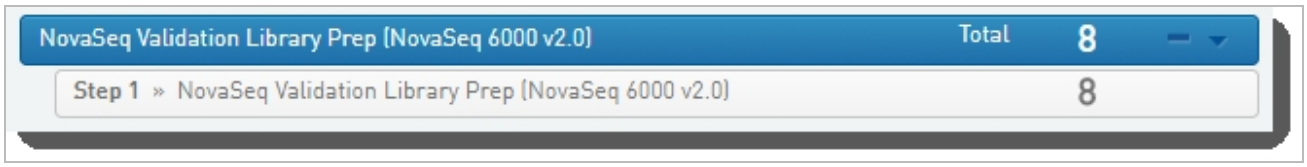
1. On the Projects and Samples screen, create a project and add samples to it.
2. Assign your samples to the NovaSeq Validation Library Prep (NovaSeq 6000 v2.0)workflow.



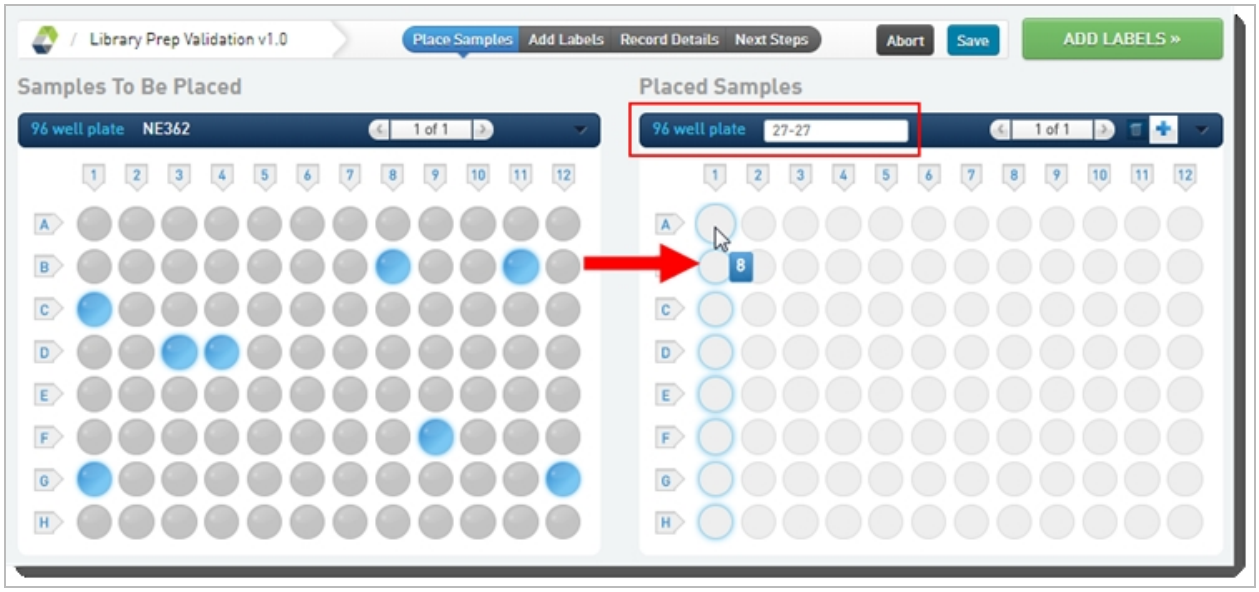
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3. Run NovaSeq Validation Library Prep (NovaSeq 6000 v2.0) step

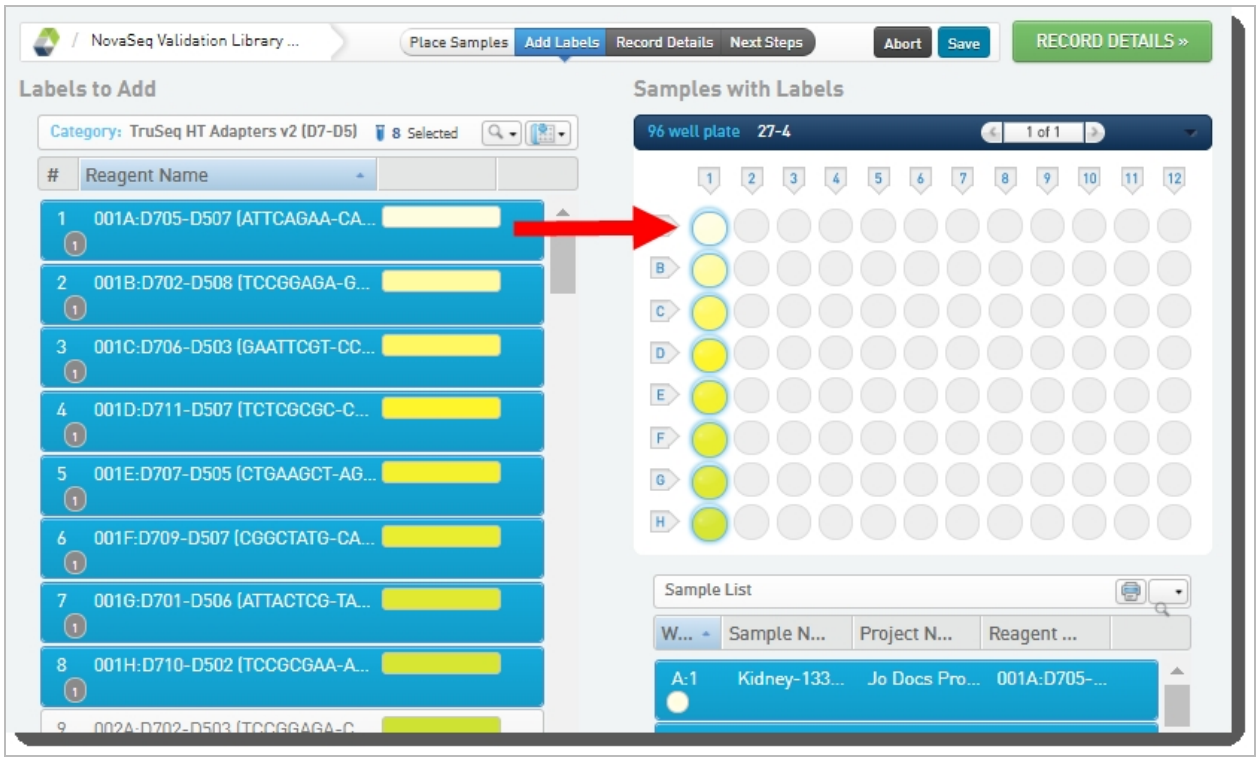
1. In Lab View, locate the NovaSeq Validation Library Prep (NovaSeq 6000 v2.0) protocol. You will see your samples queued for the NovaSeq Validation Library Prep (NovaSeq 6000 v2.0)step.



2. Add the samples to the Ice Bucket and begin work.
3. On the Ice Bucket screen, in the Reagent Label Options panel, select a group of reagent labels from the drop-down list and click Begin Work.
4. On the Placement screen:
   a. In the Placed Samples area on the right, in the container field, scan or enter the barcode of the destination container.
   b. Select the samples from the Samples to be Placedarea on the left, and then drag them over to the container wells on the right.



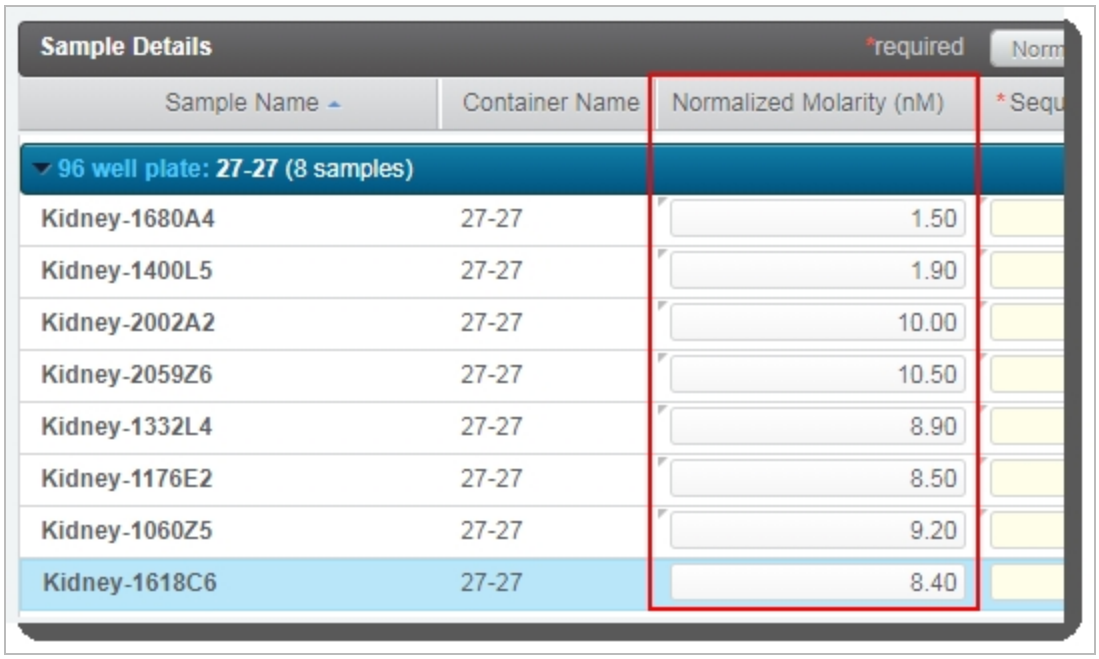
   c. Click Add Labels.
5. On the Add Labels screen, the Labels to Add list is populated with labels from the TruSeq HT Adapters v2 (D7-D5) label group. This is the only label group configured in the out-of-the-box integration and it is used by default in this step.
   a. Select reagent labels on the left, and drag them over to the container on the right to assign them to placed samples.



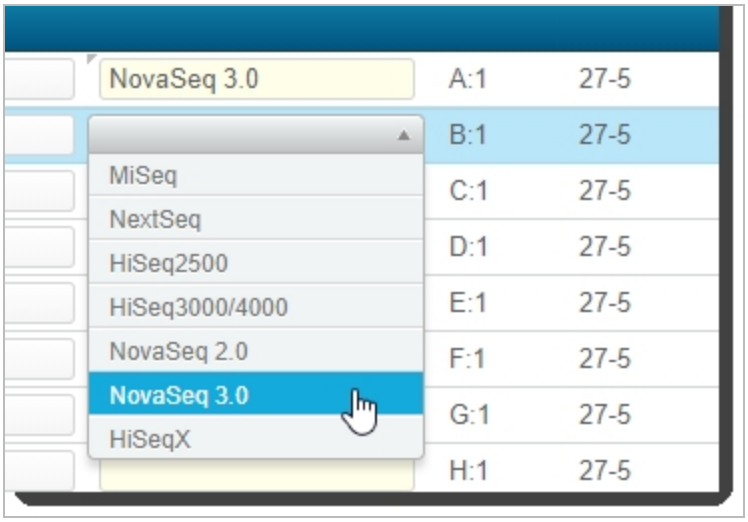
b. Click Record Details.

6. On the Record Details screen:

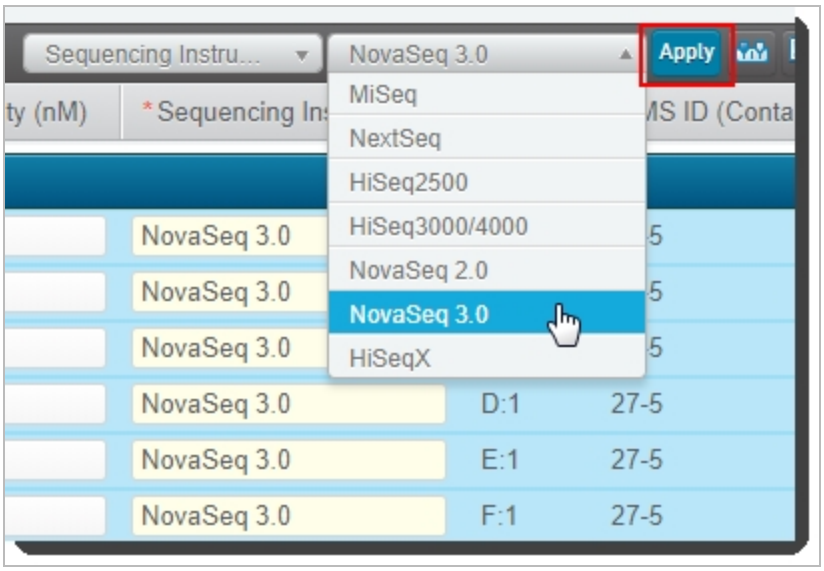
a. In the Sample Details table, enter values for Normalized Molarity. You can type values individually, or use Copy/Paste functionality to enter multiple values from a spreadsheet (see note below).



b. The Sequencing Instrument column indicates the instrument to be used for the sequencing run. Select NovaSeq 3.0.
   You can populate the fields in this column using one of the following methods:
   - To populate fields individually for each sample:
     - Select from the drop-down list in each row.

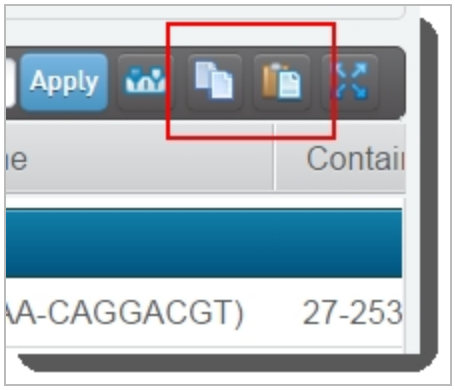


   - To populate fields all at once:
     - Use Copy/Paste to enter multiple values from a spreadsheet (see Note below).
     - Alternatively, click inside the table and press Ctrl + A to select all rows. At the top of the table, select Sequencing Instrument and then select NovaSeq 3.0 from the adjacent drop-down list. Click Apply.



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If you are working with a large number of samples, you can use Copy / Paste functionality to enter multiple values from a spreadsheet.

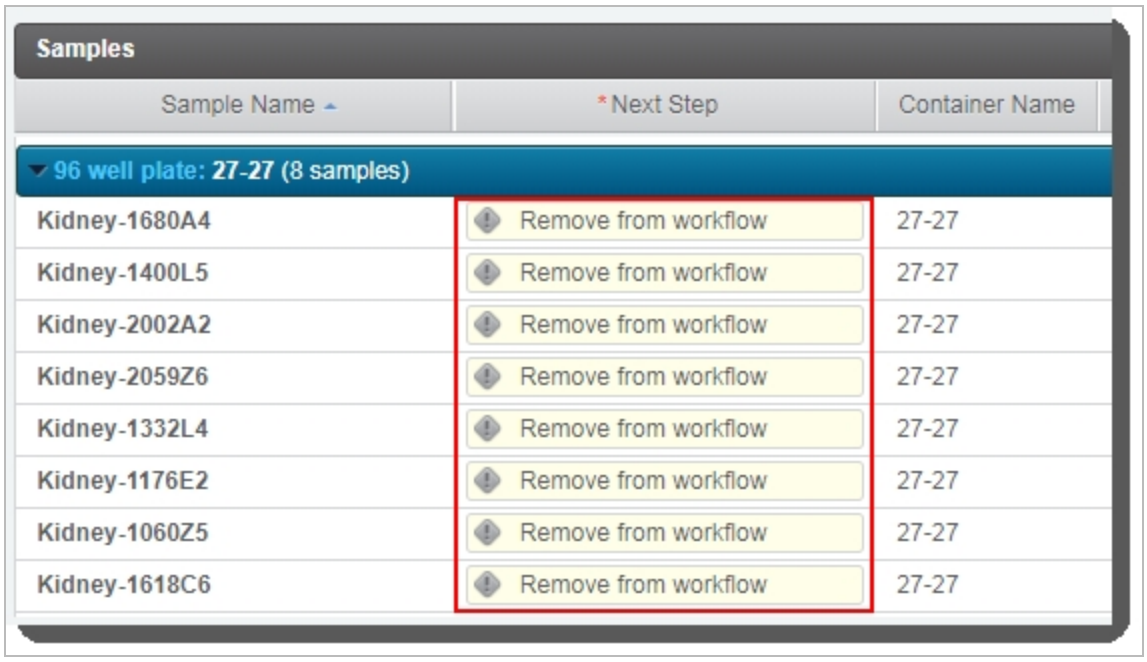


1. Click the Copy Samples button to copy the sample information from the LIMS to the Clipboard.
2. Open a blank Excel document and paste the contents of the Clipboard into the spreadsheet.
3. In the spreadsheet, enter the Normalized Molarity values. Select the entire spreadsheet, including the headers (Ctrl + A), and copy (Ctrl + C) the information to the Clipboard.
4. In the LIMS, click the Paste Samples button. Paste the contents of the clipboard (Ctrl + V) into the Update Table window and then click Update to paste into the Sample Details table.

5. Click Next Steps.
   - This triggers the Next Steps automation, which sets the value of the next step (for all samples) to Remove from workflow. Note that the Routing Script automation expects this value and requires it in order to successfully advance samples to the next step.
6. On the Assign Next Steps screen, in the Samples table, the Next Step for all samples is pre-populated with Remove from workflow.

   Do not change this value! If Next Step is not set to Remove from workflow, the routing script will not be able to route samples correctly.

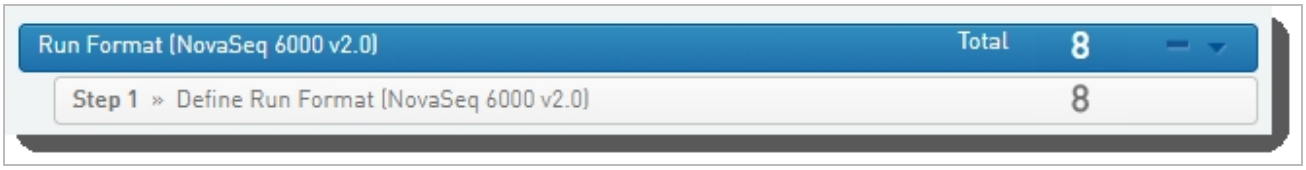
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7. Click Finish Step.
   On exit from the step, the Routing Script automation is triggered. This automation assigns samples to the first step of the NovaSeq 6000 v2.0 workflow - Define Run Format (NovaSeq 6000 v2.0). This is the only step in Protocol 1: Run Format (NovaSeq 6000 v3.0).

4. Run Define Run Format (NovaSeq 6000 v2.0) step

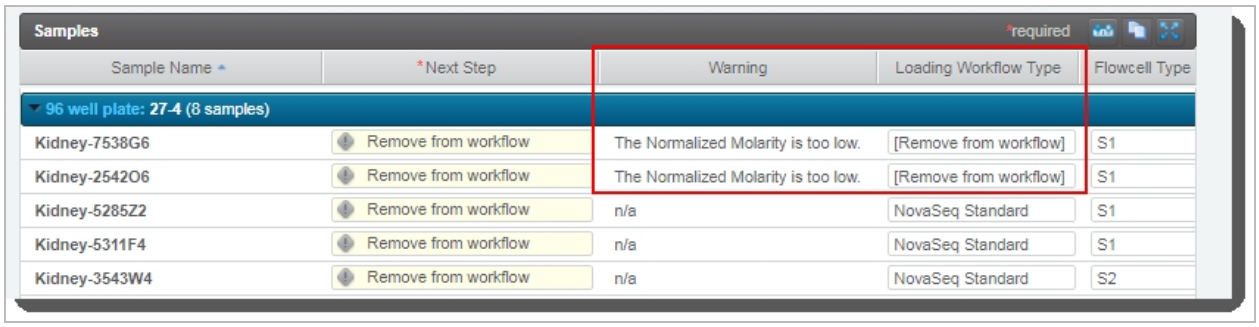
1. In Lab View, locate the Run Format (NovaSeq 6000 v2.0) protocol. You will see your samples queued for the Define Run Format (NovaSeq 6000 v2.0)step.



2. Add the samples to the Ice Bucket and begin work.
3. On the Record Details screen, in the Sample Details table, note the following fields:Loading Workflow Type: As this version of the integration only supports the standard workflow, this field is pre-populated by the LIMS with the value NovaSeq Standard.
   - Normalized Molarity: These values are copied over from the previous step. (If the user did not populate this column during library prep, they can enter the values here.)
4. Populate the following fields (values can vary across samples):Flowcell Type: Select S1, S2, or S4.
   - Final Loading Concentration (pM): You may select from the two preset options - 225 (for PCR-free workflows) or400 (for Nano workflows), or you may enter a different value.
5. Click Next Steps. This triggers the Next Steps automation, which does the following: Sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value, and requires it in order to successfully advance samples to the next step.

- Calculates the Minimum Molarity.
- Checks Normalized Molarity value. For samples with no Normalized Molarity value (i.e., empty value, not including 0), generates an error message informing the user that the field cannot be empty.
- Compares each sample's Normalized Molarity value with the Minimum Molarity value.

6. On the Assign Next Steps screen: In the Sample Details table, the Next Step for all samples is pre-populated with Remove from workflow - regardless of the Loading Workflow Type. Do not change this value! If Next Step is not set to Remove from workflow, the routing script will not be able to route samples correctly.
    - For samples whose Normalized Molarity value was found to be lower than the Minimum Molarity value, the Loading Workflow Type is set to Remove from workflow and a message is recorded in the Warning field for the sample.

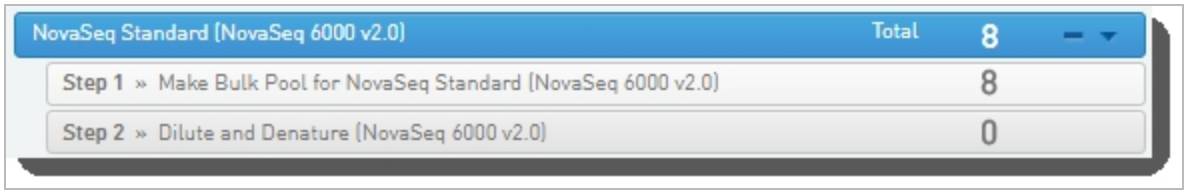


- At this point you have two options: Return to the Record Details screen and adjust the Normalized Molarity value so that it equals or exceeds the Minimum Molarity value. You will also need to set the Loading Workflow Type to NovaSeq Standard.
- Complete the protocol without correcting the Normalized Molarity value. In this case, the samples in question will be removed from the LIMS workflow.

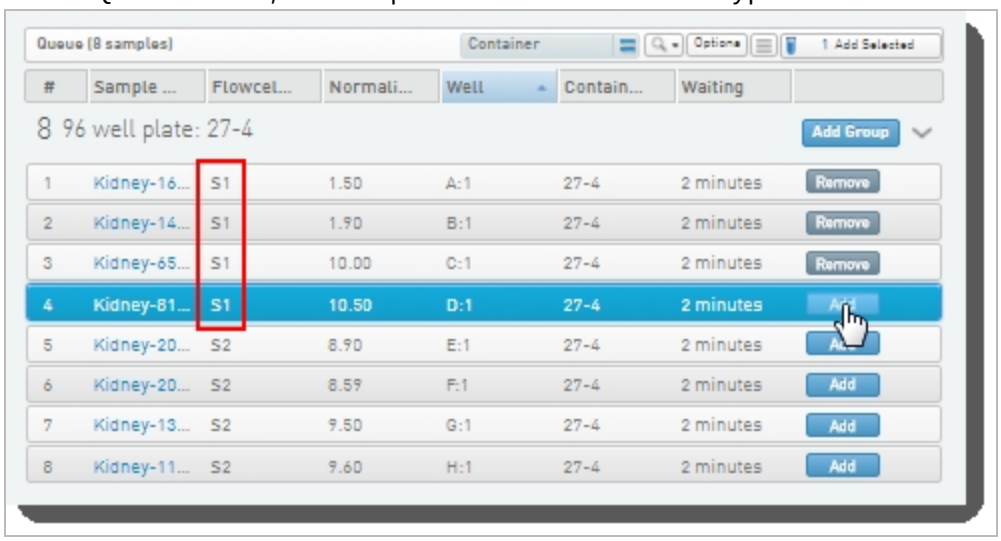
7. Click Finish Step. The Routing Script automation is triggered: Samples whose Loading Workflow Type is set to Remove from workflow (i.e., whereNormalized Molarity value is lower than the Minimum Molarity) are removed from the LIMS workflow.
    - Samples whose Loading Workflow Type is NovaSeq Standard are routed to the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0) step. This is the first of two steps in the NovaSeq Standard (NovaSeq 6000 v2.0) protocol.

5. Run Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0) step

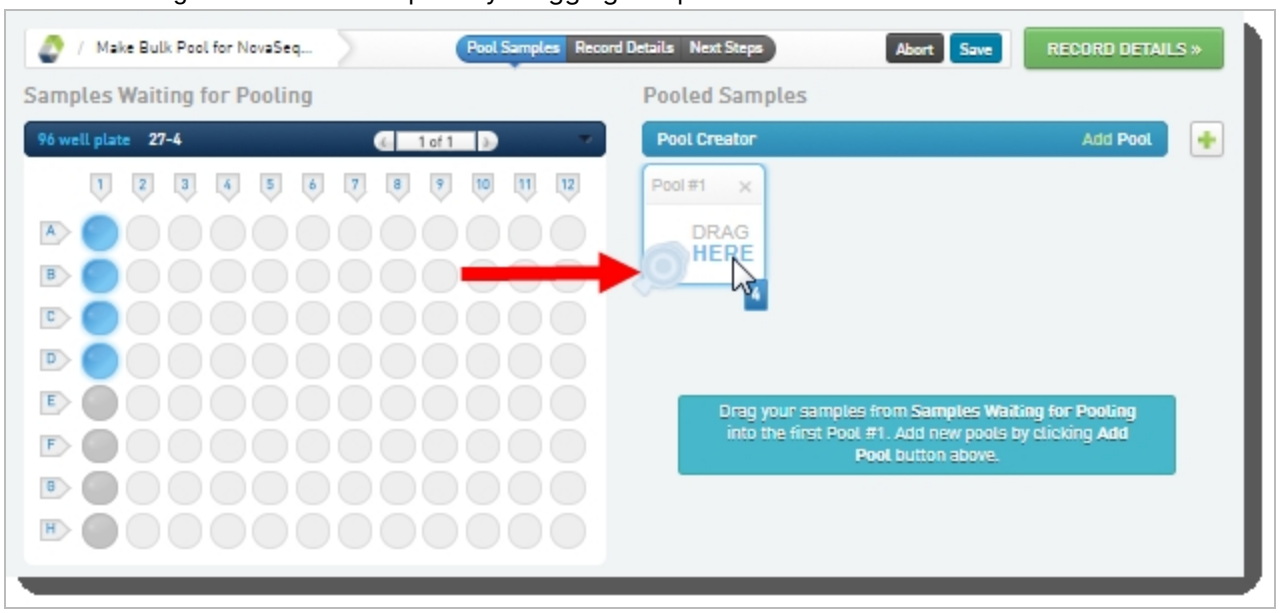
1. In Lab View, locate the NovaSeq Standard (NovaSeq 6000 v2.0) protocol. You will see your samples queued for the Make Bulk Pool for NovaSeq Standard (NovaSeq 6000 v2.0)step.



2. On the Queue screen, add samples of the same Flowcell Type to the Ice Bucketand begin work.

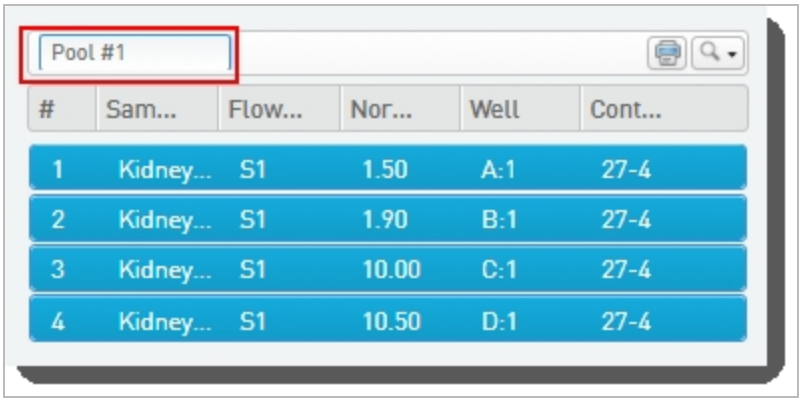


3. On the Pooling screen: Create a pool by dragging samples into the Pool Creator



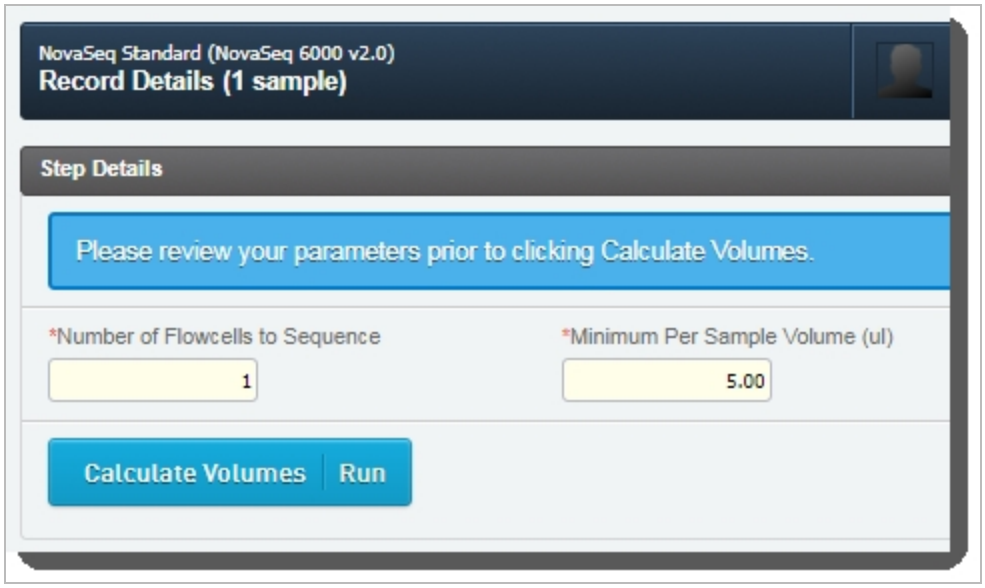
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- You can type a name for the pool, or accept the default name - Pool #1.



- Click Record Details.

4. On exit from the Pooling screen, the Validate Inputs Flowcell Type and Single Pool is triggered. The automation checks that: All samples in the pool have been assigned the same Flowcell Type.
    - Only one pool has been created.
5. On the Record Details screen, the Step Details area contains two required fields:Number of Flowcells to Sequence: The value entered in this field is used in volume calculations, to ensure that volumes are sufficient for the number of times the pool will be sequenced.
    - Minimum Per Sample Volume (ul): The value in this field is used to calculate how much of each sample will be included in the pool. The field is pre-populated with the configured default value - 5 ul, but may be edited if required.

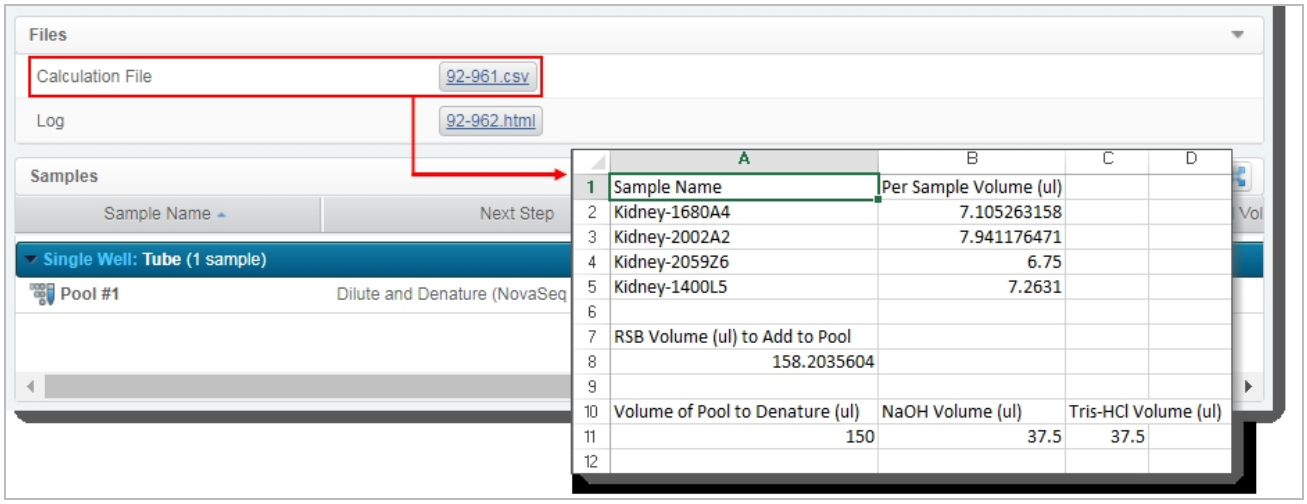


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Note:
Assuming the default Minimum Per Sample Volume (ul) value of 5, for a given batch:
1. If the smallest Per Sample Volume (ul) value is less than 5, the LIMS automatically assigns a value of 5 to the sample's Adjusted Per Sample Volume (ul) field.
2. The LIMS then adjusts the Adjusted Per Sample Volume (ul) field value for all other samples in the batch, based on the ratio used to increase the lowest value to 5.

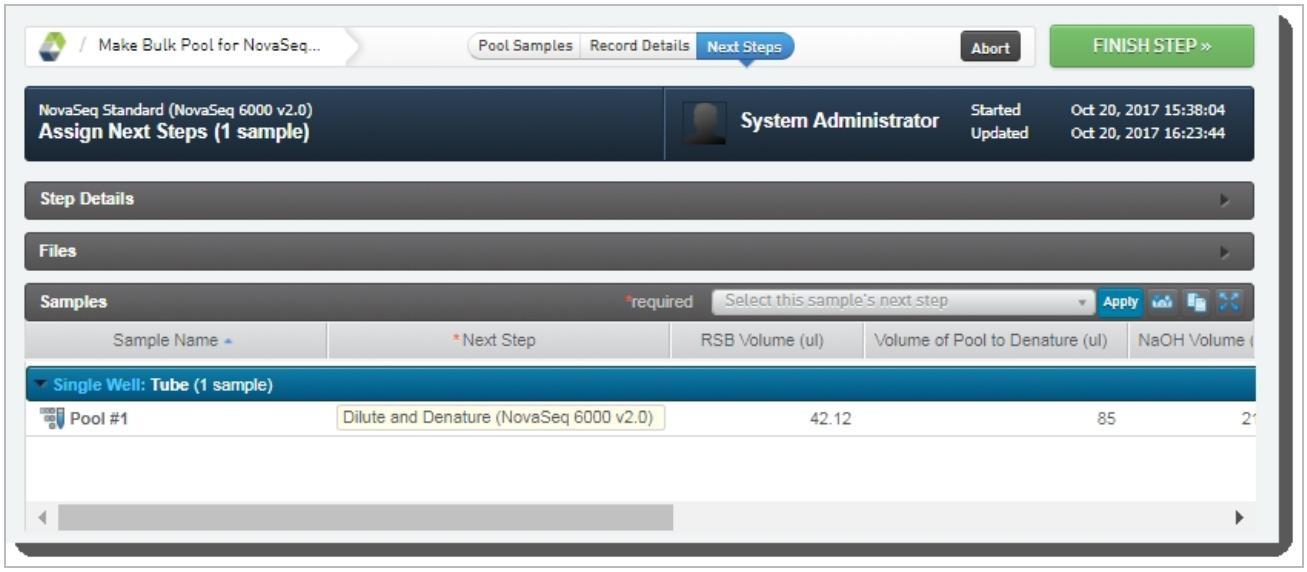
- In the Sample Details table, you can click on the pool icon to view details on the pool composition.
- Click Calculate Volumes. This triggers the Calculate Volumes automation, which performs volume calculations based on the selected Flowcell Type : Calculates Bulk Pool Volume (ul) value.

– Calculates the Per Sample Volume (ul) value to be added to the pool.
– Calculates the Total Sample Volume (ul) value.
– If the Total Sample Volume is less than the Bulk Pool Volume, calculates the RSB Volume (ul) value.
– Populates the Flowcell Type and Loading Workflow Type columns of the Sample Details table.
– Populates the Volume of Pool to Denature (ul), NaOH Volume (ul) and Tris-HCl Volume (ul) columns of the Sample Details table. (Values are set by a script and are not editable by the user.)
– The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains volume information about the pool and the individual samples it contains. Click on the file to download it and then open it in Excel.



- Click Next Steps. This triggers the Set Next Step automation. This automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol - Dilute and Denature (NovaSeq 6000 v2.0).

6. On the Assign Next Steps screen, the next step for samples is already set to the next step in the workflow - Dilute and Denature (NovaSeq 6000 v2.0).

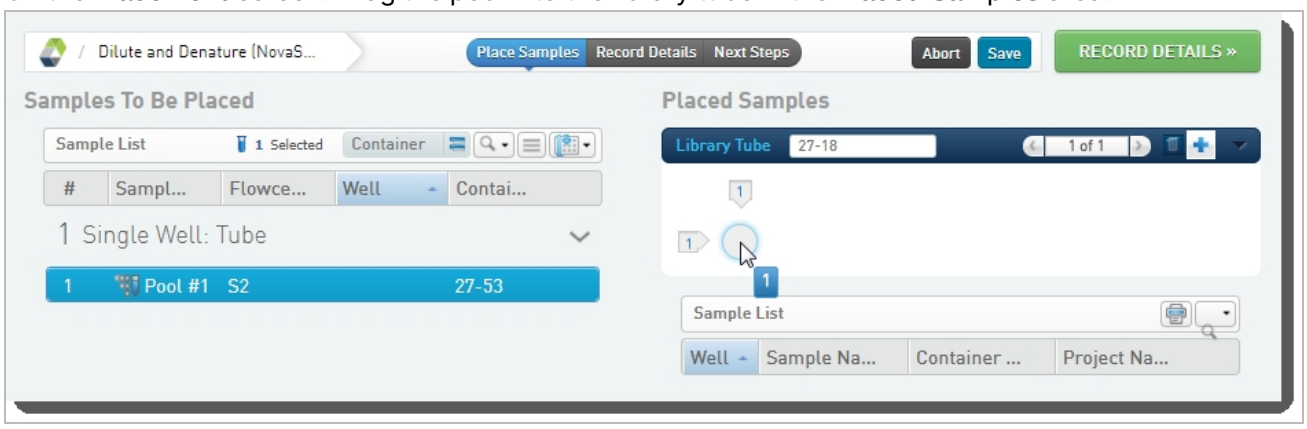


Click Finish Step.

At the end of this step, the pool of samples automatically advances to the Dilute and Denature (NovaSeq 6000 v2.0) step.

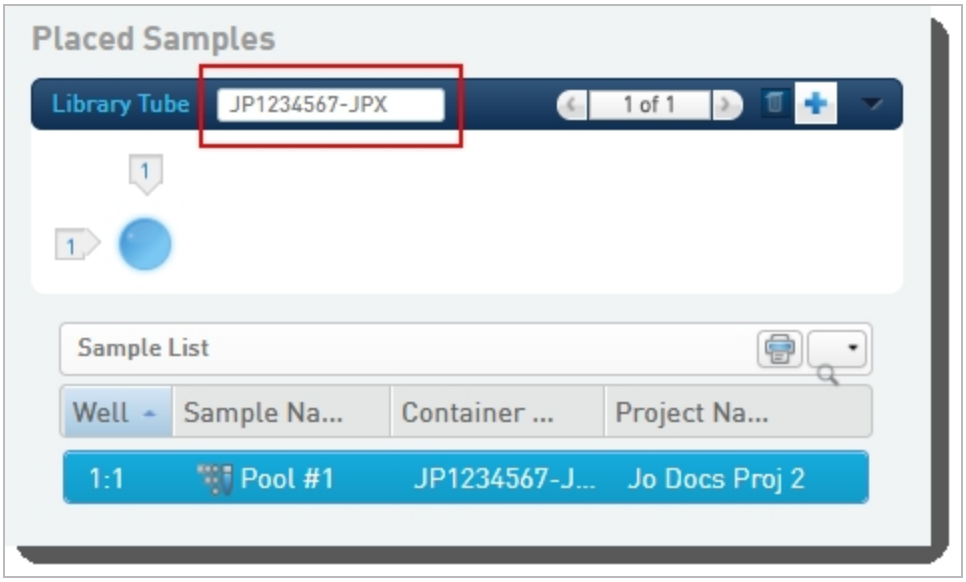
6. Run Dilute and Denature (NovaSeq 6000 v2.0) step

1. In Lab View, locate the NovaSeq Standard (NovaSeq 6000 v2.0) protocol. You will see the pool of samples queued for the Dilute and Denature (NovaSeq 6000 v2.0) step.
2. Add the samples to the Ice Bucket and begin work. At the beginning of the step, the Validate Single Input automation is triggered. This automation checks that there is only one container input to the step.
3. On the Placement screen: Drag the pool into the library tube in the Placed Samples area.



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- Scan or type the barcode of the library tube into the Library Tubefield.

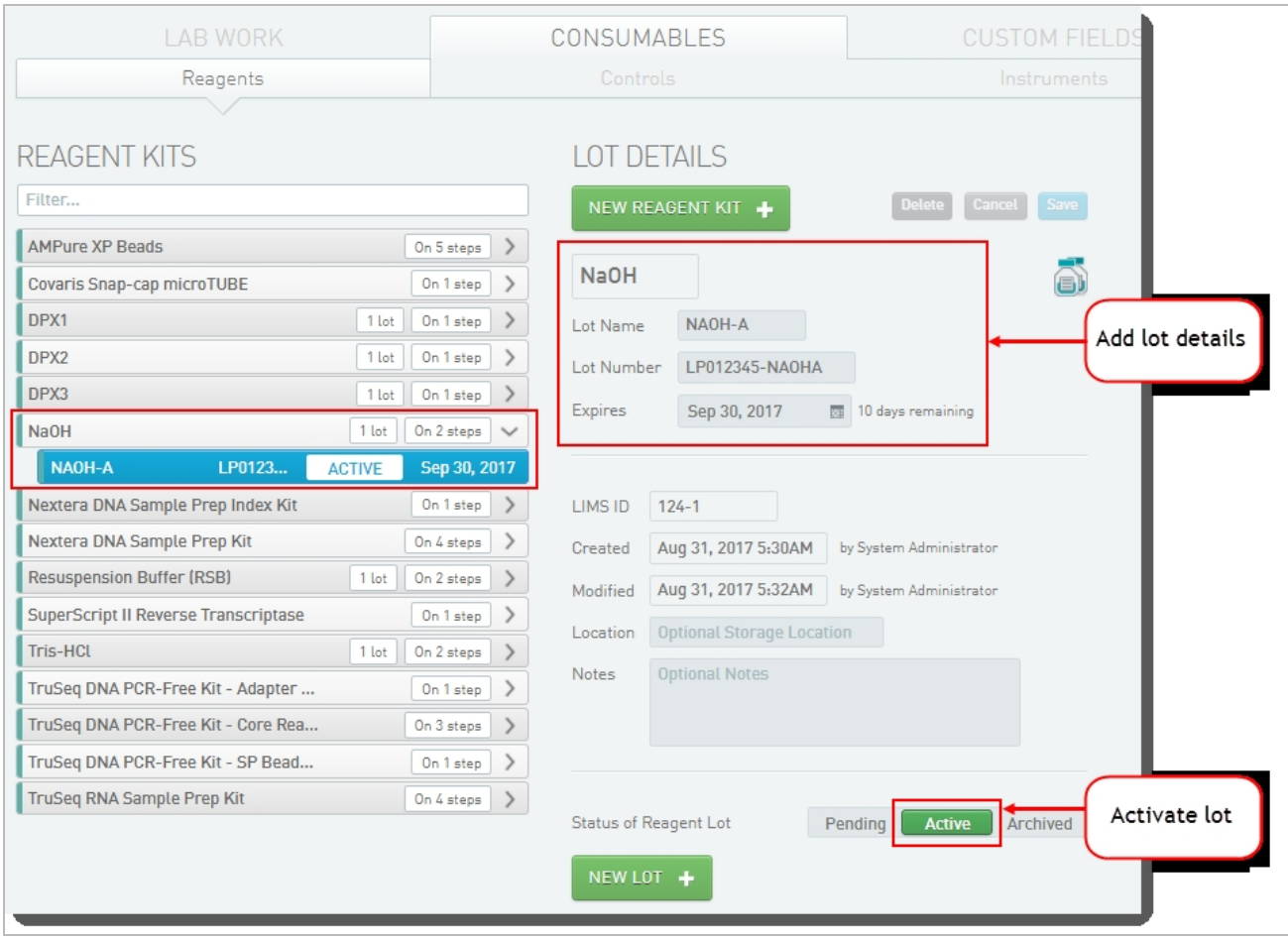


- Click Record Details.On exit of the Placement screen, the Validate Library Tube Barcode automation checks that the library tube barcode conforms to the barcode mask [A-Za-z]{2}[0-9]{7}-[A-Za-z]{3}, and displays an error message if this is not the case.

4. On the Record Details screen, the Reagent Lot Tracking section lets you track the NaOH, Resuspension Buffer, and Tris-HCI reagents used in the step. You will need to add and activate lots for these reagents. To add and activate reagent lots:
    - In a new browser tab, open an additional instance of the LIMS. Navigate to the Configuration > Consumables tab.
    - Select the NaOH reagent kit and click New Lot.
    - Enter the lot details and click Save.

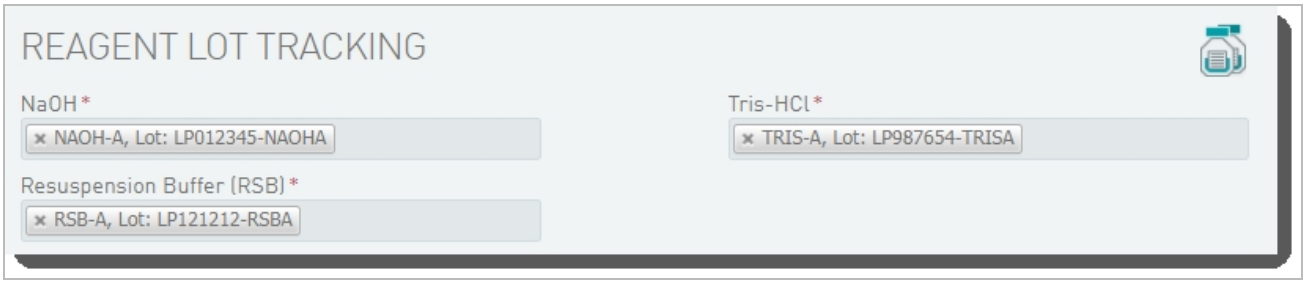
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- Set the Status of Reagent Lot toggle switch to Active.



- Repeat this process to add and activate Resuspension Buffer and Tris-HCl reagent lots.
- Close this browser tab and return to your original working tab. Refresh the page.

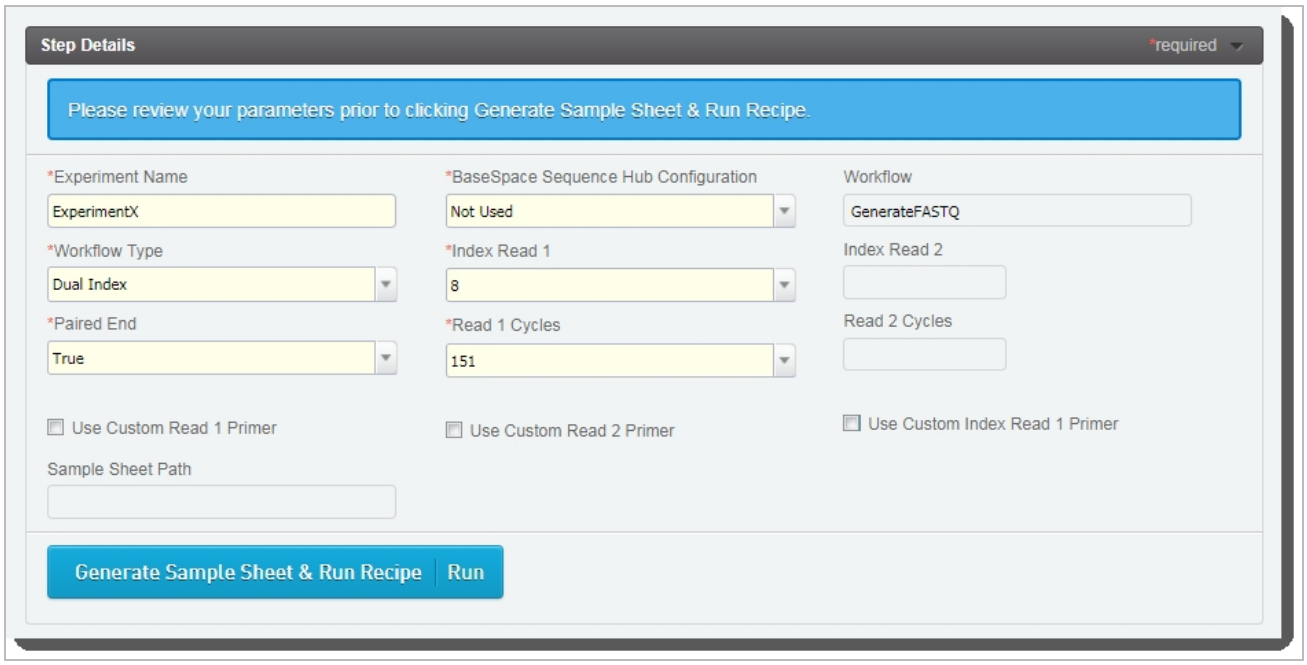
5. In the Reagent Lot Trackingsection, select from the active lots displayed in each drop-down list.



The fields displayed in the Step Details section are used to generate the sample sheet and run recipe files. Some of these fields are auto-populated and some must be completed by the user (see

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table below, for details).



Fields displayed on Record Details screen of Denature and Dilute (NovaSeq 6000) 1.0 step

Field	Value
Experiment Name	Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. No spaces.
BaseSpace Sequence Hub Configuration	Select from preset options: Not Used, Run Monitoring Only, or Run Monitoring and Storage
Workflow Type	Select from preset options: No Index, Single Index, Dual Index, or Custom
Index Read 1	Select from preset options: 0, 6, or 8 - or type a value between 0 and 20.
Index Read 2	If Dual Index workflow type selected, once the sample sheet has been generated, Index Read 2 value is automatically populated to match Index Read 1.
Paired End	Select from preset options: True or False
Read 1 Cycles	Select from preset options: 151, 101, or 51 - or type a value between 1 and 151.
Read 2 Cycles	If Paired End = True, once the sample sheet has been generated, Read 2 Cycles value is automatically populated to match Read 1 Cycles.
Use Custom Read 1 Primer	Select if applicable.
Use Custom Read 2 Primer	Select if applicable.
Use Custom Index Read 1 Primer	Select if applicable.

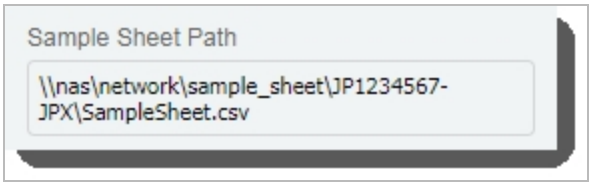
For Research Use Only. Not for use in diagnostic procedures.

Field	Value
Sample Sheet Path	Automatically populated once the sample sheet has been generated.
Workflow	Automatically populated with preset - GenerateFASTQ.

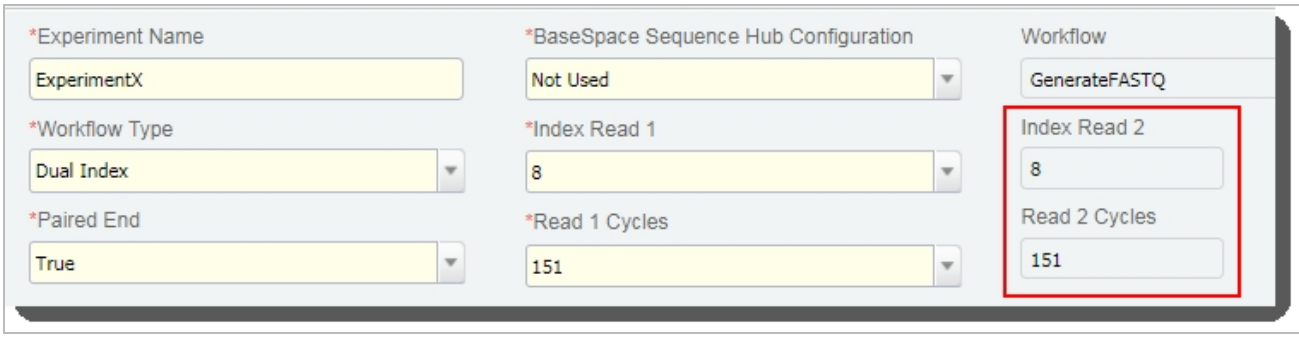
6. On the Record Details screen, click Generate Sample Sheet and Run Recipe . This triggers the automation script: The sample sheet and JSON files are generated and attached to the placeholders in the Files area of the Record Detailsscreen.



   - The Sample Sheet Pathfield is populated with the path to the sample sheet file.



   - If applicable, the Index Read 2 and Read 2 Cycles fields are populated.



   - Click Next Steps. This triggers the Prepare Files for NovaSeq automation which does the following:Copies the sample sheet and run recipe files to the location specified during installation. The NovaSeq instrument software uses these files to set up the run.

   – Sets the value of the next step to Remove from workflow. The Routing Script automation expects this value, and requires it in order to successfully advance samples to the next step.

7. On the Assign Next Steps screen: The Next Step for samples is pre-populated with Remove from workflow. ⚠Do not change this value! If Next Step is not set to Remove from workflow, the routing script will not be able to route samples correctly.

   - Click Finish Step.

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8. On exit from the step: The Routing Script automation is triggered and samples are routed to the AUTOMATED NovaSeq Run (NovaSeq 6000 v2.0) protocol.
    - In Lab View, the pool of samples is queued for the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) step.

At this point in the workflow, the user interaction ends. For details, see the *NovaSeq 6000 Integration v2.1 - User and Configuration Guide*.

 Do not add samples to the Ice Bucket or start the AUTOMATED NovaSeq Run (NovaSeq 6000 v2.0) step. The integration will do this automatically.

7. Review data parsed by AUTOMATED NovaSeq Run (NovaSeq 6000 v2.0) step

The integration starts the AUTOMATED NovaSeq Run (NovaSeq 6000 v2.0) step automatically and data from the run is parsed back into the LIMS.

No user interaction is required. However, you may open and review the various stages of the step in the LIMS.

How the integration works

1. When the user initiates a run on the NovaSeq instrument, the NovaSeq Control Software (NVCS) looks for the sample sheet and run recipe (*.json file) on the shared network drive.The NVCS copies the sample sheet to the run directory.
    - The NVCS uses the run recipe to start the sequencing run.
2. When the run starts: The instrument software creates a new run folder on the shared network drive and copies the following files into that folder:RunParameters.xml
    - RunInfo.xml
    - LIMS/ <libraryID>.json file (run recipe)
        - The Real-Time Analysis v3 (RTA3) software creates the InterOp folder.
3. The sequencing service detects the presence of the RunParameters, RunInfo, and JSON files and starts the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) step in the LIMS.
4. As the run progresses, the InterOp data files are filled in.
5. At the end of the run, the NovaSeq creates the CopyComplete.txt file in the run folder, which indicates to the sequencing service that the run has completed and the data files are ready.
6. The NovaSeq copies the run data files to the InterOp folder and the sequencing service triggers the Read InterOp Metrics automation, which reads the files, records the parsed metrics into the LIMS, and finally completes the step in LIMS.

Parse Run Parameters and Generate Link to Run Folder automation

The Parse Run Parameters and Generate Link to Run Folder automation is automatically triggered on entry to the Record Details screen. This automation parses the RunParameters.xml file attached on the Step Setup screen and populates the step UDFs / custom fields with extracted values. For details on this automation, including how the parsed fields map to the step UDFs / custom fields, see the *NovaSeq 6000 Integration v2.1 - User and Configuration Guide*.

Read InterOp Metrics automation

Automatically triggered when the sequencing service detects the CopyComplete.txt file has been created, this automation parses the InterOp data generated by the run.

The read_interop_metrics script records summary metrics into the following global UDFs / custom fields in the LIMS. The script will fail if any field is not configured or cannot be written to.

- Yield PF (Gb) R1
- Yield PF (Gb) R2
- Reads PF (M) R1
- Reads PF (M) R2
- Cluster Density (K/mm^2) R1
- Cluster Density (K/mm^2) R2
- %PF R1
- %PF R2
- % Bases >=Q30 R1
- % Bases >=Q30 R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- % Aligned R1
- % Aligned R
- % Error Rate R1
- % Error Rate R2

For more details, see the *User and Configuration Guide*.

Set Next Steps automation

This automation sets the next step for all samples to Mark protocol as complete. For more details, see the *User and Configuration Guide*.

Troubleshooting

If an automation trigger does not appear to run its corresponding scripts, see the following articles:

- *Troubleshooting Automated Informatics* in the Clarity LIMS documentation, Automated Informatics section
- *Troubleshooting EPP / Automation* in the API > Clarity LIMS API Overview > API Advanced Topics section

If an error occurs that does not provide direction on how to proceed, complete the following steps:

1. Confirm the version of the installed Illumina NovaSeq Integration Package by running the following command:

```
rpm -qa | grep -i novaseq
```

    - For an on-premise installation, run the command on the LIMS server command line. This will retrieve the version of both RPMs installed.
    - For a Software as a Service (SaaS) installation, run the command on your local server installation. This will retrieve the version of the remote extensions RPM installed.
2. If the error is related to the AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0) step, there are two places to check for log file information:
    - If the step does not start, check the NovaSeqIntegrator.log log file written to:

```
/opt/gls/clarity/extensions/novaseq/SequencingService/NovaSeqIntegrator.log
```

    - If the step starts but does not complete, open the step in the LIMS (it can be found via a search). On the Record Details screen, the log file will be attached to a placeholder called Log File. If you are unable to reach the Record Details screen (for example, if the step stopped execution on the Step Setup screen), or if the file does not contain an error, review the NovaSeqIntegrator.log described above.
3. Contact the BaseSpace Clarity LIMS support team, supplying the relevant information from the troubleshooting steps already performed.

Additional troubleshooting information is provided on the Illumina Integrations FAQ page.

Illumina NovaSeq 6000 Integration v2.1 Release Notes

- BaseSpace Clarity LIMS v4.2 or later, though not compatible with v6.0 and later
- NGS Extensions Package v5.15 or later
- BaseSpaceLIMS-AutomationWorker 0.1.0 (LIMS v4.2) or v1.0 (LIMS v5.0)

Illumina NovaSeq Software Compatibility: NovaSeq Control Software (NVCS) v1.2.0

Features to note for this release

This release of the BaseSpace Clarity LIMS integration with the Illumina NovaSeq 6000 system is significant as it provides the following:

- An updated workflow with support for:S1*, S2, and S4 flow cells per sample
  - Different applications on the same flow cell
  - Sample and reagent calculations based on flow cell
  - Underlying compatibility and logic for the upcoming single lane loader
- A run recipe (*.json file) that interacts with the latest NovaSeq 6000 instrument software

* S1 flow cell will be released in the near future

Known issues

There are no known issues for this release.

Documentation

For detailed information related to this release, see the following documentation:

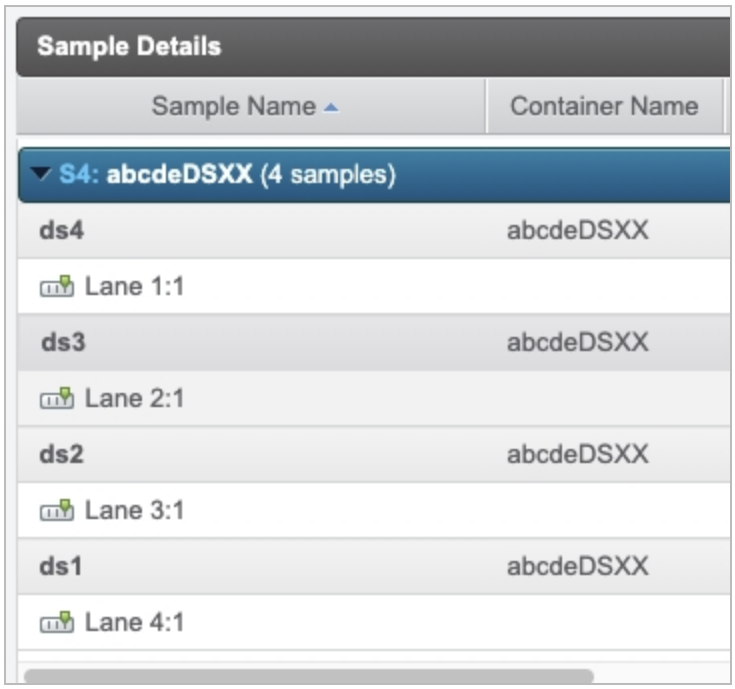
- Illumina NovaSeq 6000 Integration - Installation Guideline (SaaS)
- Illumina NovaSeq 6000 Integration - User and Configuration Guide
- Illumina NovaSeq 6000 Integration - Validation and Troubleshooting Guide

Migrate NovaSeq 6000 v2.0 Configuration to Display the Correct Lane Number

Recommended Usage: For users implementing the NovaSeq 6000 v2.0 workflow.

This solution provides a way to fix the incorrect lane number display without any software upgrades, by using the "InputWellLocation" token instead of "OutputItemNumber." However, it is not a perfect solution, since the sample details will display both container row and column numbers.

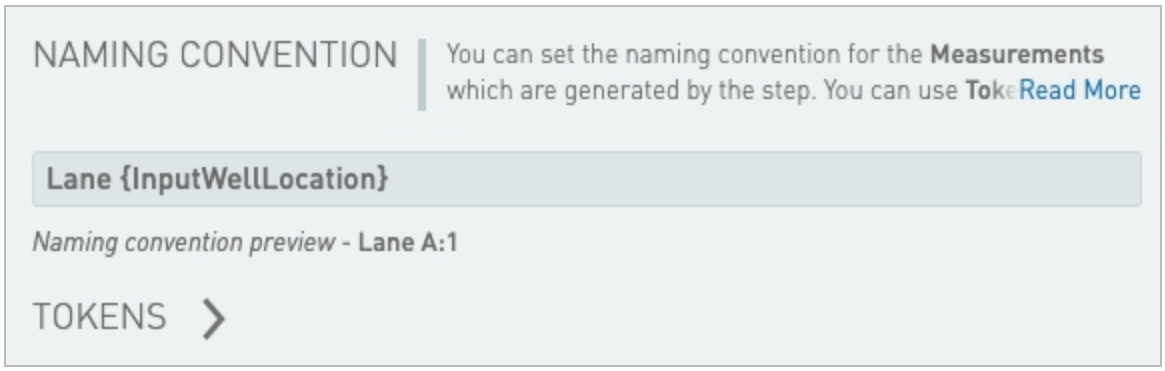
Here is an illustration of the result:



Instructions

To resolve this issue, you must update the “Naming Convention” configuration for master step: AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0).

1. Log into BaseSpace Clarity LIMS with System Administrator permissions.
2. In the Configuration area, click LAB WORK, and then choose master step AUTOMATED - NovaSeq Run (NovaSeq 6000 v2.0).
3. In Naming Convention, change the value to `Lane {InputWellLocation}`.



4. Click Save.

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NextSeq 1000/2000 Integrations

The documents in this section support the Illumina NextSeq 1000/2000 Integrations. These integrations are compatible with Illumina cloud hosted deployments only.

NextSeq 1000/2000 On-Premise Integration

This section contains the following information for the on-premise integration of the NextSeq 1000/2000 instrument:

- Installing the on-premise integration
- Configuring Clarity LIMS for the NextSeq 1000/2000 on-premise instrument
- Using Clarity LIMS to perform workflow steps
- Validating the NextSeq 1000/2000 integration
- Troubleshooting any issues related to the integration
- Release notes

NextSeq 1000/2000 On-Premise Integration v1.0 Configuration and User Interaction

The Illumina NextSeq 1000/2000 On-Prem Integration Package v1.0 supports the on-premise integration of Clarity LIMS to Illumina NextSeq 1000/2000 sequencing systems.

For instructions on validating and troubleshooting the NextSeq 1000/2000 Integration, refer to *NextSeq 1000/2000 On-Prem Integration v1.0 Validation and Troubleshooting* on page 1409.

The configuration provided in this integration has been established to support NextSeq 1000/2000 lab processes. Any configuration changes to protocols or workflows (including renaming protocols, steps, and fields) could break the process.

Overview

The integration includes the following features:

- Preconfigured NextSeq 1000/2000 On-Prem Sequencing v1.0 workflow that maps to lab protocols and instrument runs.
- The following preconfigured protocols:
    - Library Pooling and Dilution (NextSeq 1000/2000 On-Prem Sequencing v1.0)
    - Load To Reagent Cartridge (NextSeq 1000/2000 On-Prem Sequencing v1.0)
    - AUTOMATED - Sequencing Run (NextSeq 1000/2000 On-Prem Sequencing v1.0)

    - Demultiplexing (NextSeq 1000/2000 On-Prem Sequencing v1.0)
- Automated generation of a sample sheet. The sample sheet is used to start the sequencing run on NextSeq 1000/2000 Control Software (NCS) via Local run mode.
- Automated tracking of the local sequencing run and parsing of run statistics into Clarity LIMS.
- Automated tracking of local secondary analysis using DRAGEN (up to BCL Convert only) and parsing of demultiplexing metrics into Clarity LIMS. The local BCL Convert is supported up to v4.2.7.
- [Optional] Preconfigured Library Prep Validation v2.3.3 workflow used for validation purposes only. The workflow contains a single-step protocol that models the library prep workflow required to produce libraries tagged with index sequences. At the end of the step, these libraries are routed to NextSeq 1000/2000 On-Prem Sequencing v1.0 workflow. For more information, refer to *NextSeq 1000/2000 On-Prem Integration v1.0 Validation and Troubleshooting* on page 1409.

Prerequisites and Assumptions

It is assumed that samples entering the NextSeq 1000/2000 On-Prem Sequencing v1.0 workflow have gone through library preparation and quantification processes. Before they are assigned to the workflow, samples have completed the following actions:

- Samples have been accessioned into Clarity LIMS.
- Samples have been run through QC and library prep.
- Samples have the Molarity (nM) global field set to some value.
  The Calculate Volumes automation in the Library Pooling and Dilution step requires a value in the Molarity (nM) global field.

For more information on sample accessioning, refer to *Sample Accessioning* and *Upload and Modify Samples* in the Getting Started section of the Clarity LIMS Core Functionality documentation.

You can assign samples to workflows automatically, using a routing script, or manually—from the Projects & Samples dashboard. Refer to *Assign and Process Samples* in the Clarity LIMS Core Functionality documentation.

Workflows, Protocols, and Steps

The NextSeq 1000/2000 On-Prem Integration Package v1.0 includes the following workflows:

- NextSeq 1000/2000 On-Prem Sequencing v1.0
- [Optional] Library Prep Validation v2.3.3 (recommended for validation purposes)

The following table lists and describes the protocols and steps included in these workflows.

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Table 298 Illumina NextSeq 1000/2000 On-Prem Integration Package v1.0 Protocols and Steps

Protocol Name	Protocol Purpose	Steps Included in Protocol
Library Prep Validation v2.3.3 Workflow		
Library Prep Validation v2.3.3	Included for validation purposes only, this protocol models the library prep steps required to advance samples to the NextSeq 1000/2000 On-Prem Sequencing v1.0 workflow. The protocol contains a single step—Library Prep Validation v2.3.3. At the end of this step, a routing script sends the samples to the first step of the NextSeq 1000/2000 On-Prem Sequencing v1.0 workflow. The first step is Library Pooling and Dilution (NextSeq 1000/2000 On-Prem Sequencing v1.0).	1. Library Prep Validation v2.3.3
NextSeq 1000/2000 On-Prem Sequencing v1.0 Workflow		

Protocol Name	Protocol Purpose	Steps Included in Protocol
NextSeq 1000/2000 On-Prem Sequencing v1.0	This protocol models the lab processes of starting a NextSeq 1000/2000 sequencing run. Details of the steps: 1. Library Pooling and Dilution (NextSeq 1000/2000 On-Prem Sequencing v1.0) a. Samples are pooled and diluted to the final loading concentration with the help of the Calculate Volume script. 2. Load To Reagent Cartridge (NextSeq 1000/2000 On-Prem Sequencing v1.0) a. The library pool from step 1 is then ready to be loaded to the NextSeq 1000/2000 reagent cartridge. b. Run and analysis information is validated. c. Sample sheet is generated and/or planned run is created. 3. AUTOMATED - Sequencing Run (NextSeq 1000/2000 On-Prem Sequencing v1.0) a. This step is a fully automated step that is started and completed automatically after the sequencing run is started and completed on the instrument side. b. All the metadata (for example, run configuration, primary run metrics) of the sequencing run is recorded automatically. Do not add samples to the Ice Bucket or start or complete the step. The integration completes these actions automatically. c. GenerateFastQ secondary analysis is planned by default for the run. Samples continue to the next step.	1. Library Pooling and Dilution (NextSeq 1000/2000 On-Prem Sequencing v1.0) 2. Load To Reagent Cartridge (NextSeq 1000/2000 On-Prem Sequencing v1.0) 3. AUTOMATED - Sequencing Run (NextSeq 1000/2000 On-Prem Sequencing v1.0) 4. Demultiplexing (NextSeq 1000/2000 On-Prem Sequencing v1.0)

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Validation Workflow

The Library Prep Validation v2.3.3 workflow allows for validation of the system after installation is complete. For details, refer to *NextSeq 1000/2000 On-Prem Integration v1.0 Validation and Troubleshooting* on page 1409.

Step Automations

Automation details are provided in the sections following the table. All automations are required for the NextSeq 1000/2000 On-Prem Sequencing v1.0 workflows to function properly.

For details on the validation workflow, refer to *NextSeq 1000/2000 On-Prem Integration v1.0 Validation and Troubleshooting* on page 1409.

Table 299 NextSeq 1000/2000 On-Prem Sequencing v1.0 Workflow Step Automations

Protocol	Step	Automation
NextSeq 1000/2000 On-Prem Sequencing v1.0	Library Pooling and Dilution (NextSeq 1000/2000 On-Prem Sequencing v1.0)	• Calculate Volumes • Set Next Step
	Load To Reagent Cartridge (NextSeq 1000/2000 On-Prem Sequencing v1.0)	• Set Next Step • Validate Reagent Cartridge Barcode • Validate Run Setup and Create Sample Sheet • Validate Single Input
	AUTOMATED - Sequencing Run (NextSeq 1000/2000 On-Prem Sequencing v1.0)	Not applicable
	Demultiplexing (NextSeq 1000/2000 On-Prem Sequencing v1.0)	• Assign Demultiplex QC flags • Set Next Step

When the Library Prep Validation v2.3.3 workflow is imported, it includes the Illumina Universal Sample Identifier global field. This field is a text field that is reserved for CLPA support and is optional. The value of this field is not required for this integration.

Step 1: Library Pooling and Dilution (NextSeq 1000/2000 On-Prem Sequencing v1.0)

In this step, the addition of RSB dilutes pooled samples. Manually create a working pool based on the final loading concentration required.

Create one pool per step. The Calculate Volumes automation supports one pool only.

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Calculate Volumes Automation

This automation is triggered when you select Calculate Volumes on the Record Details screen. The automation completes the following actions:

- Sets RSB Volume for Pool (µl) field value to 24 for calculation purpose of the 2 nM intermediate library pool.
- Copies the Final Loading Concentration (pM) from step inputs to step outputs.

```
-exp 'step.::RSB Volume for Pool (ul):: = 24; output.::Final Loading
Concentration (pM):: = step.::Final Loading Concentration (pM)::'
```

- Calculate per sample volume required for each library to make the 2 nM intermediate library pool (24 µl in volume).

```
-exp 'input.::Per Sample Volume (ul):: = ( 48/ input.::Molarity (nM):: );
step.::RSB Volume for Pool (ul):: -= input.::Per Sample Volume (ul)::'
```

- Calculate the volume required of the 2 nM library pool to be diluted further to the Final Loading Concentration (pM) with the Final Loading Volume (µl).

```
-exp 'step.::Library Pool Volume (ul):: = (step.::Final Loading Concentration
(pM):: * step.::Final Loading Volume (ul)::/2000); output.::RSB Volume (ul)::
= step.::Final Loading Volume (ul):: - step.::Library Pool Volume (ul)::'
```

- Uses the `NextSeq1K2K_Pool1.csv`, `NextSeq1K2K_Pool2.csv`, and `NextSeq1K2K_Pool3.csv` template files to generate a single CSV file containing information about the pool and the samples it contains. The generated file is stored in the Calculation File placeholder, in the Files section, for download.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NextSeq1K2K_Pool1.csv
\
-o 1.csv \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NextSeq1K2K_Pool2.csv
\
-o 2.csv \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NextSeq1K2K_Pool3.csv
\
-o 3.csv \
&& cat 1.csv 2.csv 3.csv > {compoundOutputFileLuid0}.csv
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol. The next step is Load to Reagent Cartridge (NextSeq 1000/2000 On-Prem Sequencing v1.0):

```
nextStep = ::ADVANCE::
```

Master Step Fields

The following fields are defined on the Library Pooling and Dilution (NextSeq 1000/2000 On-Prem Sequencing v1.0) step.

Table 300 Library Pooling and Dilution (NextSeq 1000/2000 On-Prem Sequencing v1.0) Master Step Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Final Loading Concentration (pM)	Numeric Dropdown	Required Field Custom Entries	Presets • 650 • 750 • 1000 • 2000
Final Loading Volume (µl)	Numeric	Required Field	Default • 24
Library Pool Volume (µl)	Numeric	Read Only	Hidden ⚠ This field is used by the Calculate Volumes automation. Removing, renaming, or modifying the field will break the automation.

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
RSB Volume for Pool (µl)	Numeric	Read Only	Hidden ⚠ This field is used by the Calculate Volumes automation. Removing, renaming, or modifying the field will break the automation.

Global Fields

The following table lists the global fields that are configured to display on the Library Pooling and Dilution (NextSeq 1000/2000 On-Prem Sequencing v1.0) step.

Table 301 Library Pooling and Dilution (NextSeq 1000/2000 On-Prem Sequencing v1.0) Global Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Final Loading Concentration (pM)	Numeric Dropdown	Required Field Custom Entries	Decimal places displayed = 0 Presets • 225 • 400
RSB Volume (µl)	Numeric	Read Only	Decimal places displayed = 2

User Interaction

Refer to *NextSeq 1000/2000 On-Prem Integration v1.0 Validation and Troubleshooting* on page 1409 for details.

Step 2: Load to Reagent Cartridge (NextSeq 1000/2000 On-Prem Sequencing v1.0)

In this step, scan the reagent cartridge barcode into Clarity LIMS, then manually place the working pool into the reagent cartridge for the NextSeq 1000/2000 run. This step validates the run setup and analysis information and generates the sample sheet file.

The NextSeq 1000/2000 reagent cartridges support different read cycle numbers. Make sure that the read cycle values configured are within the maximum allowable reads for the cartridge type.

Validate Single Input Automation

Automatically triggered at the beginning of the step, this automation does the following actions:

- Checks that there is only one container input to the step.

```
script:validateSampleCount -min 1 -max 1
```

Validate Reagent Cartridge Barcode Automation

Automatically triggered on exit of the Placement screen, the following automation validates the reagent cartridge barcode to make sure it conforms to the barcode mask [A-Z]{2}[0-9]{7}-[A-Z0-9]{4}:

```
if (!output.container.name.matches( ::[A-Z]{2}[0-9]{7}-[A-Z0-9]{4}:: ) )
{fail ( ::Invalid Reagent Cartridge Barcode. Please verify and try again.:: ) }
```

Validate Run Setup and Create Sample Sheet Automation

Automatically triggered when a button on the Record Details screen is selected, this automation does the following actions:

- Validates the parameters entered on the Record Details screen. These parameters are used to set up the run and generate the sample sheet file.
  - Run Name can only contain alphanumeric, dash, underscore, or period characters. Spaces are not permitted.

    ```
    if (!step.::Run Name::.matches(::[a-zA-Z0-9-_.]+::))
    { fail(::Run Name contains prohibited characters. Allowed characters are:
    a-z, A-Z, 0-9, -, _, and .::) }
    ```

  - Run Name must not exceed 255 characters.

    ```
    if (step.::Run Name::.length() > 255) { fail(::Run Name shall not exceed
    255 characters.::) };
    ```

- Checks the Index Reads, Index Read 1, and Index Read 2 field values.
  - If Index Reads is No Index, Index Read 1 and Index Read 2 values must be 0 (error results if it is otherwise).

  - If Index Reads is Single Index, Index Read 1 value must be greater than 0, and Index Read 2 values must be 0 (error results if it is otherwise).
  - If Index Reads is Dual Index, Index Read 1 and Index Read 2 values must be greater than 0 (error results if it is otherwise).

```
if (step.::Index Reads:: == ::No Index::)
{ if (step.::Index Read 1:: != 0 || step.::Index Read 2:: != 0) { fail
(::Index Read 1 and Index Read 2 must be 0 if the Index Reads is No
Index.::) } }
else
{ if (step.::Index Reads:: == ::Single Index::)
{ if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) { fail
(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if the
Index Reads is Single Index.::) } }
else
{ if (step.::Index Read 1:: == 0 || step.::Index Read 2:: == 0) { fail
(::Index Read 1 and Index Read 2 must be greater than 0 if the Index Reads
is Dual Index.::) } } }
```

- Checks the Paired End, Read 2 Cycles, and Index Read 2 field values.
  - If Paired End is set to True and Read 2 Cycles value is 0, an error is generated.
  - If Paired End is set to False and Read 2 Cycles or Index Read 2 values are greater than 0, an error is generated.

```
if (step.::Paired End::.toBoolean())
{ if (step.::Read 2 Cycles:: == 0) { fail(::Read 2 Cycles must not be zero
if it is Paired End read.::) }}
else
{if (step.::Read 2 Cycles:: != 0 || step.::Index Read 2:: != 0) {fail
(::Read 2 Cycles and Index 2 Cycles must be 0 if it is not Paired End
Read.:: )}}
```

- Checks the Adapter Sequence Read 1 and Adapter Sequence Read 2 field values.
  - Adapter Sequence Read 1 and Adapter Sequence Read 2 can only contain ACTG+ characters.

```
if (step.hasValue(::Adapter Sequence Read 1::) && !step.::Adapter Sequence
Read 1::.matches(::[ACTG+]+::)) { fail(::Adapter Sequence Read 1 contains
prohibited characters. Allowed characters are: ACTG+::) };
if (step.hasValue(::Adapter Sequence Read 2::) && !step.::Adapter Sequence
Read 2::.matches(::[ACTG+]+::)) { fail(::Adapter Sequence Read 2 contains
prohibited characters. Allowed characters are: ACTG+::) }
```

- Checks Override Cycles field value.
  - Override Cycles can only contain Y, N, I, U, 0–9, and semicolon characters.

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```
if (step.hasValue(::Override Cycles::) && !step.::Override
Cycles::.matches(::[YNIU0-9;]+::))
{ fail(::Override Cycles contains prohibited characters. Allowed
characters are: Y, N, I, U, 0-9 and ;. Example: N1Y150;I8;I7N1;Y141U10.::)
}
```

- Checks Local Analysis Workflow Versions field value.
  - Local Analysis Workflow Versions can only contain 0–9 and period characters. This field must start and end with numbers that are separated by a period (for example, 3.8.4).

```
if (!step.::Local Analysis Workflow Version::.matches
(::^\\d+\\.\\d+\\.\\d+$::)) { fail(::Local Analysis Workflow Version
contains prohibited characters. Allowed characters are 0-9 and period. It
shall start and end with numbers and separated by single period e.g.
3.8.4::) };
```

- Generates the sample sheet.
  - Sample sheet is attached to the step and is also copied to the sample sheet directory that is configured during installation.
  - Refer to *NextSeq 1000/2000 On-Prem Integration v1.0 Validation and Troubleshooting* on page 1409 for submitted sample name character restrictions.

    Sample sheet validation in the Load to Reagent Cartridge step must only have alphanumeric, dash, and underscore characters in the submitted sample name. Any other characters are replaced with an underscore. The Sample Details table in the Demultiplexing step reflects the modified submitted sample name.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NextSeq1K2K_
OnPrem_Samplesheet.csv \
-o {compoundOutputFileLuid0}.csv \
-q true \
-destLIMSID {compoundOutputFileLuid0} \
-l {compoundOutputFileLuid2} \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p
{password} script:download_attachments \
-b 'prop:integration.nextseq1k2k-onprem.v1.sampleSheetDir' \
-s 'CONTAINER_NAME' \
-f {compoundOutputFileLuid0} \
-l {compoundOutputFileLuid1}"
```


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Set Next Step Automation

Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol—AUTOMATED - Sequencing Run (NextSeq 1000/2000 On-Prem Sequencing v1.0). The automation is automatically triggered on exit of the Record Details screen.

```
nextStep = ::ADVANCE::
```

Master Step Fields

The following fields defined on the Load to Reagent Cartridge (NextSeq 1000/2000 On-Prem Sequencing v1.0) step are required for sample sheet generation.

Table 302 Load to Reagent Cartridge (NextSeq 1000/2000 On-Prem Sequencing v1.0) Master Step Fields Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Run Name	Text	Required Field	
Instrument Type	Text Dropdown	Required Field	Presets • NextSeq1000 • NextSeq2000
Run Mode	Text	Required Field Read Only	Presets • Local
Paired End	Text Dropdown	Required Field	Presets • True • False
Read 1 Cycles	Numeric Dropdown	Required Field Custom Entries	Presets • 301 • 151 • 101 • 51 Range: 1–351 Decimal places displayed: 0

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Read 2 Cycles	Numeric Dropdown	Required Field Custom Entries	Presets • 301 • 151 • 101 • 51 Range: 0–351 Decimal places displayed: 0
Index Reads	Text Dropdown	Required Field	Presets • No Index • Single Index • Dual Index
Index Read 1	Numeric Dropdown	Required Field Custom Entries	Presets • 0 • 6 • 8 Range: 0–20 Decimal places displayed: 0
Index Read 2	Numeric Dropdown	Required Field Custom Entries	Presets • 0 • 6 • 8 Range: 0–20 Decimal places displayed: 0
Analysis Workflow	Text	Required Field Read Only	Default • GenerateFASTQ
Adapter Sequence Read 1	Text		
Adapter Sequence Read 2	Text		

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Barcode Mismatches Index 1	Numeric Dropdown		Presets • 0 • 1 • 2
Barcode Mismatches Index 2	Numeric Dropdown		Presets • 0 • 1 • 2
Override Cycles	Text		
Local Analysis Workflow Versions	Text	Required Field	
FASTQ Compression Format	Text Dropdown	Required Field	Presets • gzip (default value) • DRAGEN

Do not rename the Local Analysis Workflow Versions field. Modifying this field can break the integration.

User Interaction

Refer to *NextSeq 1000/2000 On-Prem Integration v1.0 Validation and Troubleshooting* on page 1409 for details.

Step 3: AUTOMATED - Sequencing Run (NextSeq 1000/2000 On-Prem Sequencing v1.0)

This step is fully automated.

The integration starts and completes the step automatically. Data from the run is parsed back to Clarity LIMS. No user interaction is required. In this step, the pooled samples in the reagent cartridge are sequenced on the NextSeq 1000/2000 instrument.

Do not add samples to the Ice Bucket or start or complete the step. The integration completes these actions automatically.

Master Step Fields

The following fields are defined on the AUTOMATED - Sequencing Run (NextSeq 1000/2000 On-Prem Sequencing v1.0) step. These fields are used to display the run status and sequencing run and analysis configuration parsed from the `RunParameters.xml` file of the sequencing run.

Do not modify the master step field names. This action causes the integration to break.

Run Parameters and Corresponding Clarity LIMS Step Fields

The following table shows how some of the step fields map to the fields on the `RunParameters.xml` file, and whether the field is visible on the Record Details screen.

Master Step Field	RunParameters.xml Field	On Record Details Screen
Current Cycle	Calculated based on CompletedCycles Field	Visible
Current Read	Calculated based on CompletedCycles field against PlannedCycles	Visible
Flow Cell ID	FlowCellSerialNumber	Visible
Flow Cell Lot Number	FlowCellLotNumber	Visible
Instrument Control Software Version	ApplicationVersion	Visible
Instrument ID	InstrumentSerialNumber	Visible
Output Folder	OutputFolder	Visible
Reagent Cartridge ID	CartridgeSerialNumber	Visible
Reagent Cartridge Lot Number	CartridgeLotNumber	Visible
RTA Version	RtaVersion	Visible
Run Name	ExperimentName	Visible
Secondary Analysis Workflow	SecondaryAnalysisWorkflow	Visible

Additional Master Step Fields and Values

The following table shows how the other step fields derive their values, and whether the step field is visible on the Record Details screen.

Master Step Field	RunParameters.xml Field	On Record Details Screen
Instrument Platform	NextSeq 1000/2000 Constant value	Visible
Instrument Type	One of the following options: • NextSeq1000 • NextSeq2000 Determined by the integration and run event	Visible
Run Status	One of the following options: • RunStarted • RunCompletedSuccessfully • RunAbortedByUser • RunErroredOut	Visible
Sequencing Log	Generated by the integration service as the sequencing run proceeds	Visible

Global Fields

The following global fields are used to capture the run metrics in Clarity LIMS:

- % Bases >=Q30 R1
- % Bases >=Q30 R2
- % Error Rate R1
- % Error Rate R2
- Yield (Gb) R1
- Yield (Gb) R2
- Reads PF R1
- Reads PF R2
- %PF R1
- %PF R2
- % Aligned R1
- % Aligned R2
- % Phasing R1
- % Phasing R2
- % PrePhasing R1

- % PrePhasing R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- Cluster Density R1
- Cluster Density R2

At the end of this step, the pool of samples is automatically advanced to (and queued for) the Demultiplexing (NextSeq 1000/2000 On-Prem Sequencing v1.0) step.

Step 4: Demultiplexing (NextSeq 1000/2000 On-Prem Sequencing v1.0)

This step is a semi-automated step.

The integration starts the step automatically and demultiplexing data from the GenerateFASTQ secondary analysis is parsed back to Clarity LIMS. The lab scientist reviews the demultiplexing result parsed into Clarity LIMS, assigns QC flags, and completes the step.

> Do not add samples to the Ice Bucket or start the AUTOMATED - Sequencing Run (NextSeq 1000/2000 On-Prem Sequencing v1.0) step. The integration completes these actions automatically.

Assign Demultiplexing QC Flags Automation

Automatically triggered when you select a button on the Record Details screen, this automation assigns QC flags based on the criteria set in the step fields.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -u {username} -p {password}
script:assignQCForDemuxStep -i {processURI:v2} -log {compoundOutputFileLuid1} -
qcResult {compoundOutputFileLuid2}
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation sets the next step to Advance and the samples to complete the protocol.

```
nextStep = ::ADVANCE::
```

Master Step Fields

The following fields are configured on the Demultiplexing (NextSeq 1000/2000 On-Prem Sequencing v1.0) master step.

Table 303 Master Step Field Configuration for Demultiplexing (NextSeq 1000/2000 On-Prem Sequencing v1.0) Step

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Criteria 1 - Source Data Field	Text Dropdown	Custom Entries	Presets • # Reads • # Perfect Index Reads • # One Mismatch Index Reads
Criteria 2 - Source Data Field	Text Dropdown	Custom Entries	Presets • # Reads • # Perfect Index Reads • # One Mismatch Index Reads
Criteria 1 - Operator	Text Dropdown	Custom Entries	Presets • >= • <= • = • !=
Criteria 2 - Operator	Text Dropdown	Custom Entries	Presets • >= • <= • = • !=
Criteria 1 - Threshold Value	Numeric		Valid integer value
Criteria 2 - Threshold Value	Numeric		Valid integer value
Log	Multiline Text	Read Only	
Analysis Status	Text	Read Only	

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Reagent Cartridge ID	Text	Read Only	Hidden Used for parsing demultiplexing results Do not delete, rename, or modify. These actions will break the integration.

Global Fields

The following table lists the global fields that are configured on the Demultiplexing (NextSeq 1000/2000 On-Prem Sequencing v1.0) step. These fields are used to display the demultiplexing result metrics for individual library in the sample pool.

Table 304 Global Field Configuration for Demultiplexing (NextSeq 1000/2000 On-Prem Sequencing v1.0) Step

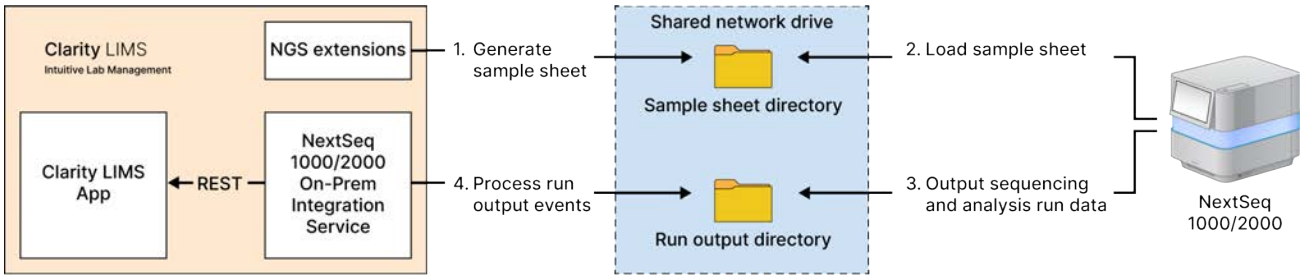
Field Name	Field Type	Field Constraints/Options
# One Mismatch Index Reads	Numeric	Read Only
# Perfect Index Reads	Numeric	Read Only
# Reads	Numeric	Read Only

User Interaction

Refer to *NextSeq 1000/2000 On-Prem Integration v1.0 Validation and Troubleshooting* on page 1409 for details.

How the NextSeq 1000/2000 On-Premise Integration Works

The following diagram shows how the integration works between the NextSeq 1000/2000 and Clarity LIMS.



Sample Sheet Generation

On the Load To Reagent Cartridge (NextSeq 1000/2000 On-Prem Sequencing v1.0) step, the run and analysis parameters entered in the Run Details screen are validated using the scripts in this step. This validation occurs when the Validate Run Setup and Create Sample Sheet automation is triggered. If the validation passes, Clarity LIMS generates the sample sheet using the driver file generator. The sample sheet contains all the run and analysis configuration required to start the run on the instrument.

For more information on how to start a local run, refer to instructions for initiating a sequence run in the *NextSeq 1000/2000 Product Documentation*.

Run Status, Primary Metrics, and Analysis Results Parsing and Recording

After the sequencing run is started on the instrument, the instrument generates the files as the sequencing and analysis run progresses. The integration monitors these files to track the run.

The following table shows the run statuses and how the integration service handles them.

Table 305 Run Status

Status	Description
RunStarted	The Run Status field is updated to RunStarted after detecting the `RunParameters.xml` file. All step custom fields are updated based on the `RunParameters.xml` file and the step field value of the Load to Reagent Cartridge step. The Multiline Sequencing Log field is updated.

Status	Description
RunCompletedSuccessfully RunErroredOut RunAbortedByUser	After detecting the `RunCompletionStatus.xml` file, the Run Status field is updated with the run status from the XML file. The Current Read and Current Cycle fields are updated to the final cycle and read numbers based on the `RunCompletionStatus.xml` file. The sequencing run result (primary metrics) is parsed from InterOp. The Multiline Sequencing Log field is updated. The integration service automatically completes the step and routes the samples in the reagent cartridge container to the Demultiplexing (NextSeq 1000/2000 On-Prem Sequencing v1.0) step.

The integration immediately starts the Demultiplexing (NextSeq 1000/2000 On-Prem Sequencing v1.0) step after completing the AUTOMATED - Sequencing Run (NextSeq 1000/2000 On-Prem Sequencing v1.0) step. In the Demultiplexing step, the integration does the following actions:

- Searches for the `CopyComplete.txt` file produced by the instrument as a sign that the analysis is complete.
  - After analysis is completed, the integration downloads the `Demultiplex_Stats.csv` file that contains the demultiplexing results. Then, the integration zips the run metric files (`Adapter_Metrics.csv`, `Demultiplex_Stats.csv`, `Index_Hopping_Counts.csv`, `Quality_Metrics.csv`, and `Top_Unknown_Barcodes.csv`) and attaches the `Run_Metrics.zip` file to the step. After detecting the `Demultiplex_Stats.csv` file, the Analysis Status field is updated to Completed. Otherwise, this field is set to Failed to signal that the BCL Convert analysis has failed.
  - The `Demultiplex_Stats.csv` file is parsed and details are recorded in the Sample Details table for each library in the library pool. For multi-lane flow cells, the demultiplex details (for example, number of reads) are aggregated as a sum.
- Updates the Multiline Sequencing Log field.

The integration does not automatically complete the step. You must assign the QC flag to the individual library before manually completing the step.

Start A Sequencing Run On Instrument

For more information on how to start a local run, refer to instructions for initiating a standard SBS or XLEAP-SBS sequencing run in the *NextSeq 1000/2000 Product Documentation*.

Components Installed

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The following sections describe the various components that are installed by default as part of this integration. These components include the following items:

- Reagent categories/label groups
- Reagent kits
- Control types
- Containers

Information on installed workflows, protocols, steps, and automation points is provided in the Workflows, Protocols, and Steps section of *NextSeq 1000/2000 On-Prem Integration v1.0 Validation and Troubleshooting* on page 1409.

Reagent Categories/Label Groups

- TruSeq HT adapters v2 (D7-D5)

Reagent Kits

- Resuspension Buffer (RSB)
- NextSeq 1000/2000 reagent cartridge

Container Types

- Tube
- 96-well plate
- NextSeq 1000/2000 reagent cartridge

Control Types

- PhiX v3

This integration supports the NextSeq 1000/2000 reagent cartridge with barcode provided in the format [A-Z]{2}[0-9]{7}-[A-Z0-9]{4} (for example, EC1234567-EC03).

Rules and Constraints

The NextSeq 1000/2000 Reagent Cartridge barcode must not be modified after a successful validation. Modifications can cause issues when Clarity LIMS tries to update the status and sample details of subsequent steps.

- The workflow configuration contains several validation checks. To make sure that the calculations work properly, it is important that you do not disable any of this validation logic. The validation checks determine:
  - Which samples, and how many, can enter each step together.

  - Which samples, and how many, can be pooled together.
- The NextSeq 1000/2000 reagent cartridge barcode must be unique. There must not be multiple NextSeq 1000/2000 reagent cartridge containers in the system with the same name.
- Reagent labels (indexes) must be unique.
- One library pool can only contain one library or control with no label (index).
- Do not manually start or complete the AUTOMATED - Sequencing Run (NextSeq 1000/2000 On-Prem Sequencing v1.0) step. This step is a fully automated step, and the integration service does not update samples correctly if they have been manually started.
- Do not manually start the Demultiplexing (NextSeq 1000/2000 On-Prem Sequencing v1.0) step. This step is semi-automated, and the SIS integration service does not update the demultiplexing results correctly if they have been manually started.

NextSeq 1000/2000 On-Prem Integration v1.0 Validation and Troubleshooting

This section explains how to validate the installation of the Illumina NextSeq 1000/2000 On-Prem Integration Package v1.0.

The validation process involves the following items:

- Running samples through the Library Prep Validation v2.3.3 workflow.
  - The workflow contains a single-step protocol that models the library prep workflow required to produce libraries tagged with index sequences. At the end of the step, these libraries are routed to NextSeq 1000/2000 On-Prem Sequencing v1.0 workflow.
- Running the libraries through the NextSeq 1000/2000 On-Prem Sequencing v1.0 workflow. This process validates the following information:
  - Successful sequential step advancement of samples in the following steps:
    1. Library Pooling and Dilution (NextSeq 1000/2000 On-Prem Sequencing v1.0)
    2. Load To Reagent Cartridge (NextSeq 1000/2000 On-Prem Sequencing v1.0)
    3. AUTOMATED - Sequencing Run (NextSeq 1000/2000 On-Prem Sequencing v1.0)
    4. Demultiplexing (NextSeq 1000/2000 On-Prem Sequencing v1.0)
  - Automatic validation of run setup information before sample sheet generation.
  - Automated generation of a sample sheet. The sample sheet is used to start the sequencing run on NextSeq 1000/2000 Control Software (NCS) via Local run mode.
  - Automated tracking of the NextSeq 1000/2000 sequencing run and parsing of run statistics to Clarity LIMS.
  - Automated tracking of the local secondary analysis using DRAGEN (up to BCL Convert only).
  - Parsing of demultiplexing metrics into Clarity LIMS.

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Before running the validation steps below, these steps assume that the NextSeq 1000/2000 On-Prem Integration Package v1.0 is installed, and that you have imported the default Clarity LIMS configuration.

For information on how the integration works, refer to *NextSeq 1000/2000 On-Premise Integration v1.0 Configuration and User Interaction* on page 1387.

Activate Workflow, Create Project, Add and Assign Samples

The following steps set up Clarity LIMS in preparation for running samples through the Library Prep Validation v2.3.3 and NextSeq 1000/2000 On-Prem Sequencing v1.0 workflows.

1. On the Configuration tab, under Workflows, activate both the Library Prep Validation v2.3.3 and NextSeq 1000/2000 On-Prem Sequencing v1.0 workflows.
   For instructions, refer to the information on configuring workflows in the Clarity LIMS Core Functionality documentation.
2. On the Projects and Samples screen, create a project and add samples to it.

   Use only alphanumeric, dash, and underscore characters in the submitted sample names. Failing to do so causes sample sheet validation failure in Load To Reagent Cartridge step.

3. Assign the samples to the Library Prep Validation v2.3.3 workflow.

Library Prep Protocol: Library Prep Validation v2.3.3

This single-step protocol models the library prep required to produce libraries tagged with index sequences that are ready for the NextSeq 1000/2000 On-Prem Sequencing v1.0 workflow.

Run Library Prep Validation v2.3.3 Step

1. In Lab View, locate the Library Prep Validation v2.3.3 protocol.
   Samples are queued for the Library Prep Validation v2.3.3 step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select the label groups to use.
   - TruSeq HT Adapters v2 (D7-D5) is the default label group delivered by Library Prep Validation v2.3.3 protocol.
   - For other reagent labels (indexes), manually create them in the Configuration screen under Consumables > Labels.

     For more information on how to create a reagent label group, refer to the *Add and Configure Labels and Label Groups* section in the Clarity LIMS Core Functionality documentation.

4. Select Begin Work.
5. On the Placement screen, perform the following actions:
   a. In the Placed Samples area in the Container field, scan or enter the barcode of the destination container.

b. Select the samples from the Samples to be Placed area, then drag them over to the container wells on the right.

c. Select Add Labels.

6. On the Add Labels screen, the Labels to Add list is populated with labels from the TruSeq HT Adapters v2 (D7-D5) label group. This label group is the only one configured in the integration. It is used by default in this step.

   a. Select labels on the left, and drag them over to the container on the right to assign them to placed samples.

   b. Select Record Details.

7. On the Record Details screen, perform the following actions:

   a. In the Sample Details table, enter values for Molarity (nM) for all the individual sample. Enter values individually or use the copy/paste functionality to enter multiple values from a spreadsheet.

      - Molarity (nM) values are used in Calculate Volume automation script in Library Pooling and Dilution (NextSeq 1000/2000 On-Prem Sequencing v1.0) step.

        If Molarity (nM) values are not entered, the Calculate Volume automation script does not work properly.

   b. The Sequencing Instrument column indicates the instrument to be used for the run. Select NextSeq 1000/2000 On-Prem.

   c. Populate the fields in this column using one of the following methods:

      - To populate fields individually for each sample, select from the drop-down list in each row.
      - To populate fields all at one time, use the Copy and Paste buttons to enter multiple values from a spreadsheet (refer to the following note).

      Alternatively, select inside the table and press Ctrl + A to select all rows. At the top of the table, select Sequencing Instrument, then select NextSeq 1000/2000 On-Prem from the adjacent drop-down list. Select Apply.

      If working with many samples, copy and paste to insert multiple values from a spreadsheet as follows.

      a. Select the Copy button to copy the sample information from Clarity LIMS to the clipboard.

      b. Open a blank Excel document and paste the contents of the clipboard into the spreadsheet.

      c. In the spreadsheet, enter the Molarity (nM) and Sequencing Instrument values. Select the entire spreadsheet, including the headers, (Ctrl + A) and copy (Ctrl + C) the information to the clipboard.

      d. In Clarity LIMS, select the Paste button. Paste the contents of the clipboard (Ctrl + V) into the Update Table window and then select Update to paste into the Sample Details table.

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8. Select Next Steps.
   This selection triggers the Next Steps automation, which sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step successfully.
9. On the Assign Next Steps screen in the Samples table, the Next Step for all samples is prepopulated with Remove from workflow.

   > Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.

10. Select Finish Step.
    On exit from the step, the Routing Script automation is triggered. This automation assigns samples to the first step of the NextSeq 1000/2000 On-Prem Sequencing v1.0 workflow, which is Library Pooling and Dilution (NextSeq 1000/2000 On-Prem Sequencing v1.0) step.

NextSeq 1000/2000 On-Prem Sequencing v1.0 Protocol

Step 1: Library Pooling and Dilution (NextSeq 1000/2000 On-Prem Sequencing v1.0)

1. In Lab View, locate the NextSeq 1000/2000 On-Prem Sequencing v1.0 protocol. The samples are queued for the Library Pooling and Dilution (NextSeq 1000/2000 On-Prem Sequencing v1.0) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Pooling screen, perform the following actions:
   a. Create a pool by dragging samples into the Pool Creator.

      > Do not create more than one pool for this step. The Calculate Volumes automation in this step supports one pool only.

   b. Type a name for the pool or accept the default name (Pool #1).
   c. Select Record Details.
      On the Record Details screen, the Reagent Lot Tracking section tracks the Resuspension Buffer (RSB) lot information used in the step.
5. In the Reagent Lot Tracking section, select from the active lots displayed in the drop-down list.
   The Step Details area contains two required fields:
   - Final Loading Concentration (pM)—The value entered in this field is the recommended final loading concentration specified in the *Illumina NextSeq 1000/2000 Sequencing System Guide* at support.illumina.com. The value depends on the library type. The default drop-down list contains the values 650, 750, 1000, and 2000. A custom value is acceptable.

- Final Loading Volume (µl)—The value in this field is the final loading volume of the pool into the reagent cartridge. The field is prepopulated with the configured default value, 24 µl, specified in the *Illumina NextSeq 1000/2000 Sequencing System Guide* at support.illumina.com. The value is editable when more volume is necessary.

6. Select Calculate Volumes.
   This selection triggers the Calculate Volumes automation. This automation calculates the volume required for each library to form a pool that has the concentration and volume specified in the step details fields.
   The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains volume information of each of the samples and RSB buffer to add to the pool. Select the file to download it, then open it in Excel.
7. In the Sample Details table, select the pool icon to view details on the pool composition.
8. Select Next Steps.
   This selection triggers the Set Next Step automation, which sets the next step for samples to ADVANCE, advancing them to the next step in the protocol. The next step is Load To Reagent Cartridge (NextSeq 1000/2000 On-Prem Sequencing v1.0).
   On the Assign Next Steps screen, the next step for samples is already set to the next step in the workflow. The next step is Load To Reagent Cartridge (NextSeq 1000/2000 On-Prem Sequencing v1.0).
9. Select Finish Step.

At the end of this step, the pool of samples automatically advances to Load To Reagent Cartridge (NextSeq 1000/2000 On-Prem Sequencing v1.0) step.

Step 2: Load To Reagent Cartridge (NextSeq 1000/2000 On-Prem Sequencing v1.0)

1. In Lab View, locate the NextSeq 1000/2000 On-Prem Sequencing v1.0 protocol.
   The pool of samples is queued for the Load To Reagent Cartridge (NextSeq 1000/2000 On-Prem Sequencing v1.0) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
   The Validate Single Input automation is triggered. This automation checks that there is only one container input to the step.
4. On the Placement screen, perform the following actions:
   a. Drag the pool into the NextSeq 1000/2000 Reagent Cartridge field in the Placed Samples area.
   b. Scan or type the barcode of the reagent cartridge into the NextSeq 1000/2000 Reagent Cartridge field.
   c. Select Record Details.

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On exit of the Placement screen, the Validate Reagent Cartridge Barcode automation checks that the reagent cartridge barcode conforms to the barcode mask [A-Z]{2}[0-9]{7}-[A-Z0-9]{4}. If not, an error message displays.

The NextSeq 1000/2000 Reagent Cartridge barcode must not be modified after a successful validation. Modifications can cause issues when Clarity LIMS tries to update the status and sample details of subsequent steps.

5. On the Record Details screen, the Reagent Lot Tracking section tracks the NextSeq 1000/2000 Reagent Cartridge lot information used in the step. Follow the steps in Library Pooling and Dilution (NextSeq 1000/2000 On-Prem Sequencing v1.0) step if you must add a lot.
6. In the Reagent Lot Tracking section, select from the active lots displayed in the drop-down list.
7. The fields displayed on the Record Details screen are used to create planned run and generate the sample sheet file.
   - Run Name—Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.
   - Instrument Type—Select from preset options (NextSeq 1000 or NextSeq 2000).
   - Run Mode—Read-only. This field is set to Local.
   - Paired End—Select from preset options (True or False).
   - Read 1 Cycles—Select from preset options (301, 151, 101, 51) or type a custom value.
   - Read 2 Cycles—Select from preset options (301, 151, 101, 51) or type a custom value.
   - Index Reads—Select from preset options (No Index, Single Index, Dual Index).
   - Index Read 1—Select from preset options (0, 6, 8) or type a custom value.
   - Index Read 2—Select from preset options (0, 6, 8) or type a custom value.
   - Analysis Workflow—Read only. This field is set to GenerateFASTQ.
   - Local Analysis Workflow Versions—This field is required. Enter the DRAGEN version of the GenerateFASTQ application installed on the instrument.
   - FASTQ Compression Format—Select gzip (default) or DRAGEN.
   - [Optional] Adapter Sequence Read 1—Enter the Read 1 adapter sequence of the index adapter kit.
   - [Optional] Adapter Sequence Read 2—Enter the Read 2 adapter sequence of the index adapter kit.
   - [Optional] Barcode Mismatches Index 1—Select from preset options (0, 1, 2). Leave it blank if you are unsure.
   - [Optional] Barcode Mismatches Index 2—Select from preset options (0, 1, 2). Leave it blank if you are unsure.

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- [Optional] Override Cycles—String used to specify UMI cycles and mask out cycles of a read (eg, N1Y150;I8;I7N1;Y141U10). Leave it blank if you are unsure.

8. On the Record Details screen, select Validate Run Setup and Create Sample Sheet.
   This selection triggers the automation script, which does the following actions:
   - Validates the parameters entered on the Record Details screen.
   - Generates the sample sheet and attaches it to the placeholder in the Files area of the Record Details screen. A copy of the sample sheet is copied to the sample sheet directory configuring during installation of the integration.
9. Select Next Steps.
   On the Assign Next Steps screen, the next step for samples is set to the next step in the workflow. The next step is AUTOMATED - Sequencing Run (NextSeq 1000/2000 On-Prem Sequencing v1.0).
10. Select Finish Step.

At the end of this step, the pool of samples automatically advances to and is queued for the AUTOMATED - Sequencing Run (NextSeq 1000/2000 On-Prem Sequencing v1.0) step. The sequencing run is ready to be started. For details on how to start the sequencing run for the local run mode, refer to *NextSeq 1000/2000 On-Premise Integration v1.0 Configuration and User Interaction* on page 1387.

Step 3: AUTOMATED - Sequencing Run (NextSeq 1000/2000 On-Prem Sequencing v1.0)

This step is fully automated.

> Do not add samples to the Ice Bucket or start or complete the step. The integration completes these actions automatically.

The integration starts the step automatically and data from the run is parsed back to Clarity LIMS. No user interaction is required. However, you can open and review the various stages of the step in Clarity LIMS. Do not perform any action when reviewing the data.

Read summary metrics are recorded for the library pool in the Step Details section and the Sample Details table.

Values are populated in the following master step fields:

- Run Name
- Current Read
- Flow Cell ID
- Reagent Cartridge Lot Number
- Instrument ID
- Run Status
- Current Cycle

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- Flow Cell Lot Number
- Instrument Platform
- Instrument Control Software Version
- Output Folder
- Secondary Analysis Workflow
- Reagent Cartridge ID
- Instrument Type
- RTA Version
- Sequencing Log

The summary metrics (per run level) populate in the following global fields.

- % Bases >=Q30 R1
- % Bases >=Q30 R2
- % Error Rate R1
- % Error Rate R2
- Yield (Gb) R1
- Yield (Gb) R2
- Reads PF R1
- Reads PF R2
- %PF R1
- %PF R2
- % Aligned R1
- % Aligned R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- Cluster Density R1
- Cluster Density R2

At the end of this step, the pool of samples automatically advances to (and queues for) the Demultiplexing (NextSeq 1000/2000 On-Prem Sequencing v1.0) step.

Step 4: Demultiplexing (NextSeq 1000/2000 On-Prem Sequencing v1.0)

This step is semi-automated.

> Do not add samples to the Ice Bucket or start or complete the step. The integration completes these actions automatically.

The integration starts the step automatically and demultiplexing data from the GenerateFASTQ secondary analysis is parsed back to Clarity LIMS. Review the data parsed and assign QC, depending on the criteria set, and complete the step.

In the Record Details screen, perform the following actions:

1. Review demultiplexing data.
   - Demultiplexing metrics are recorded for the library pool in the Sample Details table. For multi-lane flow cells (for example, P3), the metrics are aggregated by summing them across all lanes. For example, the # Reads displayed in the Sample Details table for a P3 flow cell (two lanes) is the summed value of the number of reads in Lane 1 and Lane 2 of `Demultiplex_Stats.csv`.
   - The metric files that are generated by the DRAGEN onboard are stored under the Files section as `Run_Metrics.zip`.
2. Assign QC flags to the individual samples. There are two ways of doing this step.
   a. Manually assign QC flags through the QC column in Sample Details table.
   b. Automatically assign QC flag by running Assign QC flags automation. This option is for scenarios where a huge number of libraries are involved.
   In the Step Details section, the following step fields are visible. (N is the number of criteria. You can use one or more criteria.)
      - Criteria N - Source Data Field—Select from preset options (for example, # Reads).
      - Criteria N - Operator—Select from preset options (for example, >= (greater than or equal to)).
      - Criteria N - Threshold Value—Enter the desired threshold value.
3. After filling up the criteria fields, select Assign QC flags. This selection triggers the automation script, which loops through each library in the pool and apply QC flag base on the criteria set previously.
   This automation also generates an AssignQC Result file under Files section.
4. Select Next Steps.
   On the Assign Next Steps screen, the Next Step field for all samples is prepopulated with Mark protocol as complete.
5. Select Finish Step.

At this point, the whole NextSeq 1000/2000 On-Prem Integration workflow is fully validated.

Troubleshooting

If an automation trigger does not appear to run its corresponding scripts, refer to *Troubleshooting Automation* in the Clarity LIMS API & Database documentation.

If the AUTOMATED - Sequencing Run (NextSeq 1000/2000 On-Prem Sequencing v1.0) step starts but does not finish, complete the following steps:

1. Log in to Clarity LIMS using the default user account and use one of the following methods to open the step in Clarity LIMS:
   - Method 1: In Lab View, find the step in the Recent Activities pane.
   - Method 2: Search for the step in Clarity LIMS using reagent cartridge barcode as the search term.
2. On the Record Details screen, locate the Sequencing Log field.
   The multiline text field contains logging information.

If you cannot reach the Record Details screen, or if the Sequencing Log field does not contain enough information to resolve the issue, contact the Clarity LIMS support team. Supply the relevant information from the troubleshooting steps already performed.

NextSeq 1000/2000 On-Prem Integration v1.0 Installation

The Illumina NextSeq 1000/2000 On-Prem Integration Package v1.0 supports the integration of Clarity LIMS to NextSeq 1000/2000 instruments.

Integration Overview

The NextSeq 1000/2000 On-Prem Integration Package v1.0 enables run planning through the v2 sample sheet that is generated or imported. The integration also tracks both sequencing run and GenerateFASTQ analysis run events without a connection to BaseSpace Sequence Hub or ICA.

The integration includes:

- The preconfigured NextSeq 1000/2000 On-Prem Sequencing v1.0 workflow that maps to lab protocols, instrument runs, and GenerateFASTQ analysis.
- The following preconfigured steps:
  - Library Pooling and Dilution (NextSeq 1000/2000 On-Prem Sequencing v1.0)
  - Load To Reagent Cartridge (NextSeq 1000/2000 On-Prem Sequencing v1.0)
  - AUTOMATED - Sequencing Run (NextSeq 1000/2000 On-Prem Sequencing v1.0)
  - Demultiplexing (NextSeq 1000/2000 On-Prem Sequencing v1.0)
- Automated generation of the v2 sample sheet using Driver File Generator to set up Local Run Mode sequencing and GenerateFASTQ analysis runs.
- Automated copying of the generated v2 sample sheet to a network drive location so that the instrument can import it.
- Automated tracking of the sequencing run and parsing of run statistics into Clarity LIMS, including:

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  - Progress and metrics of sequencing run
  - Sequencing run parameters
  - Reagent lot information
  - Sequencing run data directory location
  - Instrument software version
  - Real-Time Analysis (RTA) version and other run specific information
- Automated tracking of secondary analysis (only GenerateFASTQ for current version) and parsing of analysis statistics into Clarity LIMS.
- [Optional] Preconfigured Library Prep Validation v2.3.3 protocol used for validation purposes only. This workflow contains a single-step protocol that models the library prep required to produce libraries tagged with index sequences that are ready for the NextSeq 1000/2000 On-Prem Sequencing v1.0 workflow. For more information, refer to *NextSeq 1000/2000 On-Prem Integration v1.0 Validation and Troubleshooting* on page 1409.

Prerequisites

NextSeq 1000/2000 On-Prem Integration v1.0 has the following prerequisites:

- Illumina Preset Protocols (IPP) v2.8 or later, which contains the NextSeq 1000/2000 On-Prem Sequencing workflow
- NGS Package v5.25.0 or later, which contains automation scripts used by the workflow
- Clarity LIMS v6.2 or later with NAS shares and mount points

Prerequisite 1: IPP v2.8 and NGS v5.25.0 Installation

The NextSeq 1000/2000 On-Prem Integration v1.0 includes the workflows, protocols, and steps provided in IPP v2.8. The following workflows can be installed with IPP v2.8:

- NextSeq 1000/2000 On-Prem Sequencing v1.0 workflow
- [Optional] Library Prep Validation v2.3.3 workflow (recommended)

NGS v5.25.0 is also installed during the IPP installation process. For instructions, refer to *Platform software Name v2.8 Installation and User Configuration*.

Prerequisite 2: Clarity LIMS with NAS Share and Mount Points

The following NAS shares and mount points are required to enable files to be transmitted between Clarity LIMS (v6.2 or later) and the NextSeq 1000/2000 instrument.

Mount Point	Description
Sample sheet directory	The integration copies the generated sample sheet to this folder (for example, `/mnt/sample_sheet`). If the folder is also shared with the instrument, then the copied sample sheet can be imported directly from this location. The glsai users need the following permissions: • Files—Read and Write • Directories—Read, Write, and Execute
Run parent directory	This directory (for example, `/mnt/run_data`) is where run information is copied to by the instrument. Each separate sequencing run must be represented by a subdirectory within the parent directory. The glsjboss users need the following permissions: • Files—Read • Directories—Read and Execute

Installation

The NextSeq 1000/2000 On-Prem Integration Package v1.0 is distributed as the ClarityLIMS-NextSeq1k2k-OnPremise RPM package. This package must be installed on the Clarity LIMS server.

The RPM package installs the following files:

File	Location	Description
JAR file	`/opt/gls/clarity/extensions/nextseq1k2k-onprem/nextseq1k2k-integration.jar`	The main application that runs the integration service. The JAR file must not be run directly.

File	Location	Description
InterOp library files	`/opt/gls/clarity/extensions/nextseq1k2k-onprem/interop/lib/*.so`	The shared library file used to extract sequencing run metrics from run directories.
Logging configuration file	`/opt/gls/clarity/extensions/nextseq1k2k-onprem/conf/log4j2.xml`	The configuration file that controls how log messages are saved and displayed.
Configuration script	`/opt/gls/clarity/config/configure_nextseq1k2k_onprem.sh`	The script used to configure integration-related properties.
Service unit file	`/usr/lib/systemd/system/nextseq1k2k-onprem.service`	The file used by systemid to start and stop the integration service.

Use the following instructions to install the integration package.

Install RPM Package

1. Log in using the admin credentials.
2. Run the following command to install the ClarityLIMS-NextSeq1k2k-OnPremise RPM package from the repository:
   ```
   dnf install ClarityLIMS-NextSeq1k2k-OnPremise
   ```

Run Configuration Scripts

1. Make sure that you are using the glsjboss user credentials.
2. Run the following script to set properties in the Clarity LIMS database:
   `/opt/gls/clarity/config/configure_nextseq1k2k_onprem.sh`

After running this script, the following prompts display:

Prompt	Step Name	Description
Automated sequencing step base name:	AUTOMATED - Sequencing Run (NextSeq 1000/2000 On-Prem	The substring that the integration service uses to find the automated sequencing run step.
Automated demultiplexing step base name:	Demultiplexing (NextSeq 1000/2000 On-Prem	The substring that the integration service uses to find the semi-automated demultiplexing step.

For more information about the properties set by this script and how to modify them, refer to *Configuration Properties* on page 1423.

Start the Sequencing Service

1. Log in as the root or glsjboss user.
2. Run the following command to start the sequencing service:
   ```
   systemctl start nextseq1k2k-onprem
   ```
3. Run the following command to check the status of the integration service:
   ```
   systemctl status nextseq1k2k-onprem
   ```

After the sequencing service starts, the following files are created at `/opt/gls/clarity/extensions/nextseq1k2k-onprem`:

File	Description
`beginSequencingRun.dat`	Contains the list of run folders that the integration has processed in the Sequencing Started event. These run folders can be ignored.
`endSequencingRun.dat`	Contains the list of run folders that the integration has processed in the Sequencing Ended event. These run folders can be ignored.
`analysisRun.dat`	Contains the list of run folders that the integration has processed in the Analysis Started/Ended event. These run folders can be ignored.
`NextSeq1k2kIntegrator.log`	Log file for the NextSeq 1000/2000 integration service.

During the initial run of the integration service, it can take some time to scan the files in the run folders within the configured Run parent directory before events are processed.

Configuration Properties

Configuration properties can be modified using the omxProps-ConfigTool utility. Depending on the modified property, the integration service may need to be restarted for the changes to take effect.

The following configuration properties are used by the integration:

Property	Default Value	Description	Restart Required
integration.nextseq1k2k-onprem.v1.pollingInterval	180	The interval (in seconds) indicates how often the integration service checks for new files created in the Run parent directory (for example, a check for newly created files in the Run parent directory is done every 180 seconds).	Yes
integration.nextseq1k2k-onprem.v1.retryInterval	180	The interval (in seconds) that the integration service waits before trying to handle the event again when it encounters an error.	Yes
integration.nextseq1k2k-onprem.v1.maxRetryAttempts	4	The number of additional times that the integration service tries to handle an event.	Yes
integration.nextseq1k2k-onprem.v1.sampleSheetDir	`/mnt/sample_sheet`	The location of the copied generated sample sheet. This location is the same as the sample sheet directory from *Prerequisite 2: Clarity LIMS with NAS Share and Mount Points* on page 1419.	No

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Property	Default Value	Description	Restart Required
integration.nextseq1k2k-onprem-v1.runFolderParentDir	`/mnt/run_data`	The location that the integration scans for new run folders to handle run events. This location is the same as the Run parent directory from *Prerequisite 2: Clarity LIMS with NAS Share and Mount Points* on page 1419.	Yes
integration.nextseq1k2k-onprem-v1.analysis.metricsFilesToZip	Adapter_Metrics.csv, Demultiplex_Stats.csv, Index_Hopping_Counts.csv, Quality_Metrics.csv, Top_Unknown_Barcodes.csv	The comma-separated list of files that the integration service compresses and zips for upload to the Demultiplexing step in Clarity LIMS. These files must be in the same directory as the `Demultiplex_Stats.csv` file (for example, `/mnt/run_data/<run_name>/Analysis/1/Data/Reports`).	No
integration.nextseq1k2k-onprem-v1.analysis.sequenceStepBaseName	AUTOMATED - Sequencing Run (NextSeq 1000/2000 On-Prem	The substring that the integration service uses to find the automated sequencing run step.	No
integration.nextseq1k2k-onprem-v1.analysis.demultiplexStepBaseName	Demultiplexing (NextSeq 1000/2000 On-Prem	The substring that the integration service uses to find the semi-automated demultiplexing step.	No

NextSeq 1000/2000 On-Prem Integration v1.0 Release Notes

These Release Notes describe the key changes to software components for the Clarity LIMS NextSeq 1000/2000 On-Prem Integration Package v1.0.

Compatibility

This version of the integration package is compatible with the following software:

- Clarity LIMS v6.2 and later

- NextSeq 1000/2000 Instrument Control Software (NCS) v1.7.0

New Features

- The new on-premise integration for NextSeq 1000/2000 supports the following functions:
  - Automated generation of a sample sheet for use with the BCL Convert analysis software.
  - Automated tracking of the sequencing run and parsing of run statistics into Clarity LIMS.
  - Automated tracking of the local DRAGEN BCL Convert analysis (BCL Convert v4.2.7 and earlier) and parsing of demultiplexing metrics into Clarity LIMS. The metrics are aggregated for multi-lane flow cells (for example, P3).

Defect Repairs

- None

Known Issues

- Sequencing run error triggered by disk space full on instrument causes `RunParameters.xml` to be empty and the run event cannot be processed on Clarity LIMS. This error is unlikely to occur as pre-run checks on the instrument check for sufficient disk space before run is started.

Limitations

- Does not support analysis requeue.

NextSeq 1000/2000 Cloud Integrations

This section contains the following information for the cloud integrations of the NextSeq 1000/2000 instrument:

- Configuring Clarity LIMS for the NextSeq 1000/2000 instrument
- Using Clarity LIMS to perform workflow steps
- Validating the NextSeq 1000/2000 integration
- Troubleshooting any issues related to the integration
- Manually upgrading existing integrations
- Release notes

NextSeq 1000/2000 Integration v2.5 Configuration and User Interaction

The Illumina NextSeq 1000/2000 Integration Package v2.5 supports the integration of Clarity LIMS to Illumina NextSeq 1000/2000 sequencing systems.

For instructions on validating and troubleshooting the NextSeq 1000/2000 Integration, refer to *NextSeq 1000/2000 Integration v2.5 Validation and Troubleshooting* on page 1461.

The configuration provided in this integration has been established to support NextSeq 1000/2000 lab processes. Any configuration changes to protocols or workflows (including renaming protocols, steps, and fields) can break the process.

Overview

The integration includes the following features:

- Preconfigured NextSeq 1000/2000 Sequencing v2.4 workflow that maps to lab protocols and instrument runs.
- The following preconfigured protocols:
    - Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.4)
    - Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.4)
    - AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.4)
    - Demultiplexing (NextSeq 1000/2000 Sequencing v2.4)
- Automatic validation of run setup information before sample sheet generation or planned run creation.
- Automated generation of a sample sheet. The sample sheet is used to start the sequencing run on NextSeq 1000/2000 Control Software (NCS) via Local run mode.
- Automated creation of a planned run. The NCS retrieves and starts the planned run via Hybrid or Cloud run mode.
- Automated tracking of the NextSeq 1000/2000 sequencing run and parsing of run statistics from BaseSpace Sequence Hub into Clarity LIMS.
- Automated tracking of the secondary DRAGEN analysis (up to BCL Convert only) and parsing of demultiplexing metrics from BaseSpace Sequence Hub into Clarity LIMS.
- The following Cloud analysis supported applications:
    - BCL Convert v4.2.7 or earlier under BaseSpace Sequence Hub DRAGEN Analysis
    - BCL Convert v4.2.7 under ICA Workflow Session Tracking
- Local analysis supported BCL Convert v4.2.7 and earlier.
- [Optional] Preconfigured Library Prep Validation v2.3.3 workflow used for validation purposes only. The workflow contains a single-step protocol that models the library prep workflow required to produce libraries tagged with index sequences. At the end of the step, these libraries are routed to NextSeq 1000/2000 Sequencing v2.4 workflow. For more information, refer to *NextSeq 1000/2000 Integration v2.5 Validation and Troubleshooting* on page 1461.
- [Optional] An integration of Clarity LIMS Product Analytics (CLPA) that make Clarity LIMS data available to the ICA and CLPA. This integration also requires installation and configuration. For more information, refer to *Clarity LIMS Product Analytics Integration v1.3 Configuration and User Interaction* on page 2388.

Prerequisites and Assumptions

The Illumina NextSeq 1000/2000 Integration Package v2.5 is compatible with Illumina cloud hosted deployments only.

This integration requires a Personal, Professional, or Enterprise BaseSpace Sequence Hub subscription.

It is assumed that samples entering the NextSeq 1000/2000 Sequencing v2.4 workflow have gone through library preparation and quantification processes. Before they are assigned to the workflow, samples have completed the following actions:

- Samples have been accessioned into Clarity LIMS.
- Samples have been run through QC and library prep.
- Samples have the Molarity (nM) global field set to some value.
  The Calculate Volumes automation in the Library Pooling and Dilution step requires a value in the Molarity (nM) global field.

For more information on sample accessioning, refer to *Sample Accessioning* and *Upload and Modify Samples* in the Getting Started section of the Clarity LIMS Core Functionality documentation.

You can assign samples to workflows automatically, using a routing script, or manually—from the Projects & Samples dashboard. Refer to *Assign and Process Samples* in the Clarity LIMS Core Functionality documentation.

Workflows, Protocols, and Steps

The NextSeq 1000/2000 Integration Package v2.5 includes the following workflows:

- NextSeq 1000/2000 Sequencing v2.4
- [Optional] Library Prep Validation v2.3.3 (recommended for validation purposes)

The following protocols and steps are included in these workflows.

Table 306 Protocols and Steps

Protocol Name	Protocol Purpose	Steps Included in Protocol
Library Prep Validation v2.3.3 Workflow		

Protocol Name	Protocol Purpose	Steps Included in Protocol
Library Prep Validation v2.3.3	Included for validation purposes only, this protocol models the library prep steps required to advance samples to the NextSeq 1000/2000 Sequencing v2.4 workflow. The protocol contains a single step—Library Prep Validation v2.3.3. At the end of this step, a routing script sends the samples to the first step of the NextSeq 1000/2000 Sequencing v2.4 workflow. The first step is Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.4).	1. Library Prep Validation v2.3.3

NextSeq 1000/2000 Sequencing v2.4 Workflow

Protocol Name	Protocol Purpose	Steps Included in Protocol
NextSeq 1000/2000 Sequencing v2.4	This protocol models the lab processes of starting a NextSeq 1000/2000 sequencing run. Details of the steps: 1. Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.4) a. Samples are pooled and diluted to the final loading concentration with the help of the Calculate Volume script. 2. Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.4) a. The library pool from step 1 is then ready to be loaded to the NextSeq 1000/2000 reagent cartridge. b. Run and analysis information is validated. c. Sample sheet is generated and/or planned run is created. 3. AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.4) a. This step is a fully automated step that is started and completed automatically after the sequencing run is started and completed on the instrument side. b. All the metadata (for example, run configuration and primary run metrics) of the sequencing run are recorded automatically. Do not add samples to the Ice Bucket or start or complete the step. The integration completes these actions automatically. c. Generated data secondary analysis is planned by default for the run. Samples continue	1. Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.4) 2. Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.4) 3. AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.4) 4. Demultiplexing (NextSeq 1000/2000 Sequencing v2.4)

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Validation Workflow

The Library Prep Validation v2.3.3 workflow allows for validation of the system after installation is complete. For details, refer to *NextSeq 1000/2000 Integration v2.5 Validation and Troubleshooting* on page 1461.

Step Automations

Automation details are provided in the sections following the table.

For details on the validation workflow, refer to *NextSeq 1000/2000 Integration v2.5 Validation and Troubleshooting* on page 1461.

Table 307 NextSeq 1000/2000 Sequencing v2.4 Workflow Step Automations

Protocol	Step	Automation
NextSeq 1000/2000 Sequencing v2.4	Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.4)	• Calculate Volumes • Register Pools & Register Step Completed[1] • Register Step Started[1] • Set Next Step
	Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.4)	• Register Step Completed[1] • Retrieve Analysis Workflow Versions • Set Next Step • Validate Reagent Cartridge Barcode • Validate Run Setup and Create Planned Run • Validate Single Input & Register Step Started[2]
	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.4)	• Register NextSeq1k2k RunCompleted & Register Step Completed[1] • Register Step Started & Register NextSeq1k2k Run & Register NextSeq1k2k Run Association & Register NextSeq1k2k RunStarted[1]
	Demultiplexing (NextSeq 1000/2000 Sequencing v2.4)	• Assign Demultiplex QC flags • Register Analysis Completed & Register Step Completed[1] • Register Step Started[1] • Set Next Step

[1] These automations are required for CLPA support only.
[2] These automations are required for the NextSeq 1000/2000 Sequencing v2.4 workflows and contain additional logic needed for CLPA support. If you would like to remove CLPA support, contact Illumina Support.

All remaining automations are required for the NextSeq 1000/2000 Sequencing v2.4 workflows.

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Importing the Library Prep Validation v2.3.3 or NextSeq 1000/2000 Sequencing v2.4 workflows include the Illumina Universal Sample Identifier global field. This text field is reserved for CLPA support and is optional. The value is not required for this integration.

Step 1: Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.4)

In this step, the addition of RSB dilutes pooled samples. Manually create a working pool based on the final loading concentration required.

Create one pool per step. The Calculate Volumes automation supports one pool only.

Register Step Started Automation

This automation registers the start time of the step by publishing messages to CLPA through the LINK service. This automation is automatically triggered on entry to the step.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_started.groovy'
```

Calculate Volumes Automation

This automation is triggered when you select Calculate Volumes on the Record Details screen. The automation completes the following actions:

- Sets RSB Volume for Pool (µl) field value to 24 for calculation purpose of the 2 nM intermediate library pool.
- Copies the Final Loading Concentration (pM) from step inputs to step outputs.

  ```
  -exp 'step.::RSB Volume for Pool (ul):: = 24; output.::Final Loading Concentration (pM):: = step.::Final Loading Concentration (pM)::'
  ```

- Calculate per sample volume required for each library to make the 2 nM intermediate library pool (24 µl in volume).

  ```
  -exp 'input.::Per Sample Volume (ul):: = ( 48/ input.::Molarity (nM):: ); step.::RSB Volume for Pool (ul):: -= input.::Per Sample Volume (ul)::'
  ```

- Calculate the volume required of the 2 nM library pool to be diluted further to the Final Loading Concentration (pM) with the Final Loading Volume (µl).

  ```
  -exp 'step.::Library Pool Volume (ul):: = (step.::Final Loading Concentration (pM):: * step.::Final Loading Volume (ul)::/2000); output.::RSB Volume (ul):: = step.::Final Loading Volume (ul):: - step.::Library Pool Volume (ul)::'
  ```

- Uses the `NextSeq1K2K_Pool1.csv`, `NextSeq1K2K_Pool2.csv`, and `NextSeq1K2K_Pool3.csv` template files to generate a single CSV file containing information about the pool and the samples it contains. The generated file is stored in the Calculation File placeholder, in the Files section, for download.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NextSeq1K2K_Pool1.csv
\
-o 1.csv \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NextSeq1K2K_Pool2.csv
\
-o 2.csv \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/NextSeq1K2K_Pool3.csv
\
-o 3.csv \
&& cat 1.csv 2.csv 3.csv > {compoundOutputFileLuid0}.csv
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol. The next step is Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.4):

```
nextStep = ::ADVANCE::
```

Register Pools & Register Step Completed Automation

This automation is automatically triggered on exit of the step and performs the following tasks:

- Registers the pool and library information used by the Clarity LIMS workflow. Samples in the pool are assumed to have gone through library preparation.
- Registers the completion time of the step by publishing messages to CLPA through the LINK service.

This automation is only used for CLPA support.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/pooling.groovy' &&
```

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_
completed.groovy'
```

Master Step Fields

The following fields are defined on the Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.4) step.

Table 308 Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.4) Master Step Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Final Loading Concentration (pM)	Numeric Dropdown	Required Field Custom Entries	Presets • 650 • 750 • 1000 • 2000
Final Loading Volume (µl)	Numeric	Required Field	Default • 24
Library Pool Volume (µl)	Numeric	Read Only	Hidden ⚠ This field is used by the Calculate Volumes automation. Removing, renaming, or modifying the field will break the automation.

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
RSB Volume for Pool (µl)	Numeric	Read Only	Hidden ⚠ This field is used by the Calculate Volumes automation. Removing, renaming, or modifying the field will break the automation.

Global Fields

The following table lists the global fields that are configured to display on the Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.4) step.

Table 309 Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.4) Global Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Final Loading Concentration (pM)	Numeric Dropdown	Required Field Custom Entries	Decimal places displayed: 0 Presets • 225 • 400
RSB Volume (µl)	Numeric	Read Only	Decimal places displayed: 2

User Interaction

Refer to *NextSeq 1000/2000 Integration v2.5 Validation and Troubleshooting* on page 1461 for details.

Step 2: Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.4)

In this step, scan the reagent cartridge barcode into Clarity LIMS, then manually place the working pool into the reagent cartridge for the NextSeq 1000/2000 run. This step validates the run setup and analysis information and generates the sample sheet file and/or creates a planned run depending on the Run Mode selected.

Use alphanumeric, dash, or underscore characters only in the submitted sample names. Any other characters can cause sample sheet validation failure in the Load to Reagent Cartridge step. The NextSeq 1000/2000 On-Prem Sequencing workflow handles these characters in the Load to Reagent Cartridge step by replacing them with an underscore in the submitted sample name. The Sample Details table in the Demultiplexing step reflects the modified submitted sample name.

The NextSeq 1000/2000 reagent cartridges support different read cycle numbers. Make sure that the read cycle values configured for the planned run are within the maximum allowable reads for the cartridge type.

Validate Single Input & Register Step Started Automation

Automatically triggered at the beginning of the step, this automation does the following actions:

- Checks that there is only one container input to the step. This script is required for the NextSeq 1000/2000 Sequencing v2.4 workflow.
  ```
  script:validateSampleCount -min 1 -max 1
  ```
- Registers the start time of the step by publishing messages to CLPA through the LINK service. This script is only used for CLPA support.
  ```
  /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_started.groovy'
  ```

Validate Reagent Cartridge Barcode Automation

Automatically triggered on exit of the Placement screen, the following automation validates the reagent cartridge barcode to make sure it conforms to the barcode mask [A-Z]{2}[0-9]{7}-[A-Z0-9]{4}:

```
if (!output.container.name.matches( ::[A-Z]{2}[0-9]{7}-[A-Z0-9]{4}:: ) )
{fail ( ::Invalid Reagent Cartridge Barcode. Please verify and try again.:: ) }
```

Retrieve Analysis Workflow Versions Automation

Automatically triggered upon entry of the Record Details screen, this automation fetches the available analysis workflow versions and updates the preset values of both of the Local Analysis Workflow Versions and Cloud Analysis Workflow Versions custom fields.

```
/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/nextseq1k2k-
automation.jar script:retrieve_analysis_workflow_versions -i {stepURI:v2} -u
{username} -p {password} -l {compoundOutputFileLuid2}
```

Do not modify or disable the Retrieve Analysis Workflow Versions automation script. Modifying or disabling the script breaks the integration.

Validate Run Setup and Create Planned Run Automation

Automatically triggered when a button on the Record Details screen is selected, this automation validates the parameters entered on the Record Details screen. These parameters are used to set up the run and generate the sample sheet file and/or create the planned run.

- Run Name can only contain alphanumeric, dash, underscore, or period characters. Spaces are not permitted.

  ```
  if (!step.::Run Name::.matches(::[a-zA-Z0-9-_.]+::))
  { fail(::Run Name contains prohibited characters. Allowed characters are: a-
  z, A-Z, 0-9, -, _, and .::) }
  ```

- Run Name must not exceed 255 characters.

  ```
  if (step.::Run Name::.length() > 255) { fail(::Run Name shall not exceed 255
  characters.::) };
  ```

- Checks the Index Reads, Index Read 1, and Index Read 2 field values.
  - If Index Reads is No Index, Index Read 1 and Index Read 2 values must be 0 (error results if it is otherwise).
  - If Index Reads is Single Index, Index Read 1 value must be greater than 0, and Index Read 2 values must be 0 (error results if it is otherwise).
  - If Index Reads is Dual Index, Index Read 1 and Index Read 2 values must be greater than 0 (error results if it is otherwise).

    ```
    if (step.::Index Reads:: == ::No Index::)
    { if (step.::Index Read 1:: != 0 || step.::Index Read 2:: != 0) { fail
    (::Index Read 1 and Index Read 2 must be 0 if the Index Reads is No
    Index.::) } }
    else
    { if (step.::Index Reads:: == ::Single Index::)
    { if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) { fail
    (::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if the
    Index Reads is Single Index.::) } }
    else
    ```


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```
{ if (step.::Index Read 1:: == 0 || step.::Index Read 2:: == 0) { fail
(::Index Read 1 and Index Read 2 must be greater than 0 if the Index Reads
is Dual Index.::) } } }
```

- Checks the Paired End, Read 2 Cycles, and Index Read 2 field values.
  - If Paired End is set to True and Read 2 Cycles value is 0, an error is generated.
  - If Paired End is set to False and Read 2 Cycles or Index Read 2 values are greater than 0, an error is generated.

```
if (step.::Paired End::.toBoolean())
{ if (step.::Read 2 Cycles:: == 0) { fail(::Read 2 Cycles must not be zero
if it is Paired End read.::) }}
else
{if (step.::Read 2 Cycles:: != 0 || step.::Index Read 2:: != 0) {fail
(::Read 2 Cycles and Index 2 Cycles must be 0 if it is not Paired End
Read.:: )}}
```

- Checks the Adapter Sequence Read 1 and Adapter Sequence Read 2 field values.
  - Adapter Sequence Read 1 and Adapter Sequence Read 2 can only contain ACTG+ characters.

```
if (step.hasValue(::Adapter Sequence Read 1::) && !step.::Adapter Sequence
Read 1::.matches(::[ACTG+]+::)) { fail(::Adapter Sequence Read 1 contains
prohibited characters. Allowed characters are: ACTG+::) };
if (step.hasValue(::Adapter Sequence Read 2::) && !step.::Adapter Sequence
Read 2::.matches(::[ACTG+]+::)) { fail(::Adapter Sequence Read 2 contains
prohibited characters. Allowed characters are: ACTG+::) }
```

- Checks Override Cycles field value.
  - Override Cycles can only contain Y, N, I, U, 0–9, and semicolon characters.

```
if (step.hasValue(::Override Cycles::) && !step.::Override
Cycles::.matches(::[YNIU0-9;]+::))
{ fail(::Override Cycles contains prohibited characters. Allowed
characters are: Y, N, I, U, 0-9 and ;. Example: N1Y150;I8;I7N1;Y141U10.::)
}
```

- When the Run Mode is Cloud, the script does the following actions:
  - Sets Local Analysis Workflow Versions to None.
  - If the Cloud Analysis Workflow Versions is None, the script displays the following error: `Invalid option selected for Cloud Analysis Workflow Versions field.`

```
if (step.::Run Mode:: == ::Cloud::) { step.::Local Analysis Workflow
Versions:: = ::None::; if (step.::Cloud Analysis Workflow Versions:: ==
::None::) { fail(::Invalid option selected for Cloud Analysis Workflow
Versions field.::); }};
```


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  - Due to automation script limitation, a validation rule resides in the backend service to perform the following action: If Cloud Analysis Workflow Versions is 3.7.4 or later, set FASTQ Compression Format to gzip.
- When the Run Mode is Local or Hybrid, the script does the following actions:
  - Sets Cloud Analysis Workflow Versions to None.
  - If Local Analysis Workflow Versions is None, the script displays the following error message:
    ```
    Invalid option selected for Local Analysis Workflow Versions field.
    ```
    ```
    if (step.::Run Mode:: == ::Local:: || step.::Run Mode:: == ::Hybrid::) {
    step.::Cloud Analysis Workflow Versions:: = ::None::; if (step.::Local
    Analysis Workflow Versions:: == ::None::) { fail(::Invalid option selected
    for Local Analysis Workflow Versions field.::); }};
    ```
  - Due to automation script limitations, a validation rule resides in the backend service. If Local Analysis Workflow Versions is 3.7.4 or later, an error occurs if FASTQ Compression Format is None.
- Generates the sample sheet and/or creates the planned run.
  - Sample sheet is attached to the step.
    ```
    /opt/gls/clarity/bin/java -jar
    /opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/nextseq1
    k2k-automation.jar
    ```
    ```
    script:generate_v2_sample_sheet -i {stepURI:v2} -u {username} -p
    {password} -l {compoundOutputFileLuid2} -c {compoundOutputFileLuid0}
    ```
  - There is backend validation of the sample sheet content. This validation makes sure that the sample sheet is valid to set up a Local Mode run or is ready for DRAGEN applications. Refer to *NextSeq 1000/2000 Integration v2.5 Validation and Troubleshooting* on page 1461 for submitted sample name character restrictions.

Set Next Step Automation

Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol—AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.4). The automation is automatically triggered on exit of the Record Details screen.

```
nextStep = ::ADVANCE::
```

Register Step Completed

Automatically triggered on exit of the step, this automation registers the completion time of the step by publishing messages to CLPA through the LINK service. This script is only used for CLPA support.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_
completed.groovy'
```

Master Step Fields

There are 24 fields defined on the Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.4) step. These fields are required for sample sheet generation and planned run creation.

Table 310 Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.4) Master Step Fields Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Run Name	Text	Required Field	
Instrument Type	Text Dropdown	Required Field	Presets • NextSeq1000 • NextSeq2000
Run Mode	Text Dropdown	Required Field	Presets • Local • Hybrid • Cloud
Paired End	Text Dropdown	Required Field	Presets • True • False
Read 1 Cycles	Numeric Dropdown	Required Field Custom Entries	Presets • 301 • 151 • 101 • 51
Read 2 Cycles	Numeric Dropdown	Required Field Custom Entries	Presets • 301 • 151 • 101 • 51

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Index Reads	Text Dropdown	Required Field	Presets • No Index • Single Index • Dual Index
Index Read 1	Numeric Dropdown	Required Field Custom Entries	Presets • 0 • 6 • 8
Index Read 2	Numeric Dropdown	Required Field Custom Entries	Presets • 0 • 6 • 8
Analysis Workflow	Text	Required Field Read Only	Default • GenerateFASTQ
Adapter Sequence Read 1	Text		
Adapter Sequence Read 2	Text		
Barcode Mismatches Index 1	Numeric Dropdown		Presets • 0 • 1 • 2
Barcode Mismatches Index 2	Numeric Dropdown		Presets • 0 • 1 • 2
Override Cycles	Text		

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Cloud Run ID	Text	Read Only	Hidden - Used in creating planned run. Do not delete, rename, or modify. These actions will break the integration.
Use Custom Index Read 1 Primer	Toggle Switch		Default • None Set Hidden Custom primer option is currently not supported. Do not enable as it will break the integration.
Use Custom Index Read 2 Primer	Toggle Switch		Default • None Set Hidden Custom primer option is currently not supported. Do not enable as it will break the integration.

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Use Custom Read 1 Primer	Toggle Switch		Default • None Set Hidden Custom primer option is currently not supported. Do not enable as it will break the integration.
Use Custom Read 2 Primer	Toggle Switch		Default • None Set Hidden Custom primer option is currently not supported. Do not enable as it will break the integration.

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Instructions	Multiline Text	Read Only	Default • If GenerateFASTQ analysis is required, select the DRAGEN version in Cloud Analysis Workflow Versions (for Cloud run mode) or Local Analysis Workflow Versions (for Local/Hybrid run mode) custom fields.
Local Analysis Workflow Versions	Text Dropdown		Default • None
Cloud Analysis Workflow Versions	Text Dropdown		Default • None
FASTQ Compression Format	Text Dropdown	Required Field	Presets • gzip (default value) • DRAGEN • None

Do not modify the configuration of the Local Analysis Workflow Versions and Cloud Analysis Workflow Versions fields. Modifying these fields can break the integration.

User Interaction

Refer to *NextSeq 1000/2000 Integration v2.5 Validation and Troubleshooting* on page 1461 for details.

Step 3: AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.4)

This step is fully automated.

The integration starts and completes the step automatically. Data from the run is parsed back to Clarity LIMS. No user interaction is required. In this step, the pooled samples in the reagent cartridge are sequenced on the NextSeq 1000/2000 instrument.

Do not add samples to the Ice Bucket or start or complete the step. The integration completes these actions automatically.

Register Step Started & Register NextSeq1k2k Run & Register NextSeq1k2k Run Association & Register NextSeq1k2k RunStarted Automation

Automatically triggered at the beginning of the step, this automation completes the following actions:

- Registers the start time of the step.
- Registers the NextSeq 1000/2000 sequencing run configuration.
- Registers the association of the sequencing run with the actual samples by linking the Instrument Run ID with the IDs for the samples.
- Registers the time that the sequencing run starts.

The automation completes these actions by publishing messages to CLPA through the LINK service. This automation is only used for CLPA support.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/common/step_
started.groovy' && /opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/unified-product-analytics/automation/unified-
product-analytics-automation.jar script:executeUPAAutomationScript -i
{stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0} -s
'com/illumina/upa/scripts/nextseq1k2k/register_nextseq1k2k_run.groovy' &&
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/nextseq1k2k/nextseq1k2k_
associate_seqrun.groovy' && /opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/unified-product-analytics/automation/unified-
product-analytics-automation.jar script:executeUPAAutomationScript -i
{stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0} -s
'com/illumina/upa/scripts/nextseq1k2k/nextseq1k2k_seqrun_started.groovy'
```

Register NextSeq1k2k RunCompleted & Register Step Completed Automation

Automatically triggered on exit of the step, this automation completes the following actions:

- Registers the sequencing run completion time, run status, and run metrics. This script requires the Cloud Run ID field from the Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.4) step.
- Registers the completion time of the step.

The automation completes these actions by publishing messages to CLPA through the LINK service. This automation is only used for CLPA support.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/nextseq1k2k/nextseq1k2k_seqrun_completed.groovy' && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/common/step_completed.groovy'
```

Master Step Fields

The following fields are defined on the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.4) step. These fields are used to display the run status and sequencing run and analysis configuration parsed from the `RunParameters.xml` file of the sequencing run.

Do not modify the master step field names. This action causes the integration to break.

Run Parameters and Corresponding Clarity LIMS Step Fields

The following table shows how some of the step fields map to the fields on the `RunParameters.xml` file, and whether the field is visible on the Record Details screen.

Master Step Field	RunParameters.xml Field	On Record Details Screen
Current Cycle	Calculated based on CompletedCycles Field	Visible
Current Read	Calculated based on CompletedCycles field against PlannedCycles	Visible
Flow Cell ID	FlowCellSerialNumber	Visible
Flow Cell Lot Number	FlowCellLotNumber	Visible
Instrument Control Software Version	ApplicationVersion	Visible
Instrument ID	InstrumentSerialNumber	Visible
Output Folder	OutputFolder	Visible
Reagent Cartridge ID	CartridgeSerialNumber	Visible

Master Step Field	RunParameters.xml Field	On Record Details Screen
Reagent Cartridge Lot Number	CartridgeLotNumber	Visible
RTA Version	RtaVersion	Visible
Run Name	ExperimentName	Visible
Secondary Analysis Workflow	SecondaryAnalysisWorkflow	Visible
Flow Cell Mode	FlowCellMode	Hidden Do not delete, rename, or modify. These actions can break the integration.
Instrument Run ID	Derived from OutputFolder	Hidden Do not delete, rename, or modify. These actions can break the integration.
Run End Time	RunEndTime	Hidden Do not delete, rename, or modify. These actions can break the integration.
Run Start Time	RunStartTime	Hidden Do not delete, rename, or modify. These actions can break the integration.
Secondary Analysis Mode	SecondaryAnalysisMode	Hidden Do not delete, rename, or modify. These actions can break the integration.

Master Step Field	RunParameters.xml Field	On Record Details Screen
Secondary Analysis Platform Version	SecondaryAnalysisPlatformVersion	Hidden Do not delete, rename, or modify. These actions can break the integration.
SkipObdd	SkipObdd	Hidden Do not delete, rename, or modify. These actions can break the integration.

Additional Master Step Fields and Values

The following table shows how the other step fields derive their values, and whether the step field is visible on the Record Details screen.

Master Step Field	Description	On Record Details Screen
Instrument Platform	NextSeq 1000/2000 Constant value	Visible
Instrument Type	One of the following options: • NextSeq1000 • NextSeq2000 Determined from Instrument Type step field from previous Load to Reagent Cartridge step.	Visible
Run Status	One of the following options: • RunStarted • RunCompletedSuccessfully • RunAbortedByUser • RunErroredOut Set by the integration service[1]	Visible
Sequencing Log	Filled in by the integration service[1] as the sequencing run proceeds	Visible

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Master Step Field	Description	On Record Details Screen
BaseSpace Run ID	Received from SQS message in the integration service[1]	Hidden Do not delete, rename, or modify. These actions break the integration.

[1]For information on how the integration works, refer to *Run Status, Primary Metrics, and Analysis Results Parsing and Recording* in *How the NextSeq 1000/2000 Integration Works* on page 1452.

Global Fields

The following global fields are used to capture the run metrics in Clarity LIMS.

- % Bases >=Q30 R1
- % Bases >=Q30 R2
- % Error Rate R1
- % Error Rate R2
- Yield (Gb) R1
- Yield (Gb) R2
- Reads PF R1
- Reads PF R2
- %PF R1
- %PF R2
- % Aligned R1
- % Aligned R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- Cluster Density R1
- Cluster Density R2

At the end of this step, the pool of samples is automatically advanced to (and queued for) the Demultiplexing (NextSeq 1000/2000 Sequencing v2.4) step.

Step 4: Demultiplexing (NextSeq 1000/2000 Sequencing v2.4)

This step is a semi-automated step.

The integration starts the step automatically and demultiplexing data from the GenerateFASTQ secondary analysis is parsed back to Clarity LIMS. The lab scientist reviews the demultiplexing result parsed into Clarity LIMS, assigns QC flags, and completes the step.

> Do not add samples to the Ice Bucket or start or complete the step. The integration completes these actions automatically.

Register Step Started Automation

Automatically triggered at the beginning of the step, this automation registers the start time of the step by publishing messages to CLPA through the LINK service. This automation is only used for CLPA support.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_started.groovy'
```

Assign Demultiplexing QC Flags Automation

Automatically triggered when you select a button on the Record Details screen, this automation assigns QC flags based on the criteria set in the step fields.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -u {username} -p {password} script:assignQCForDemuxStep -i {processURI:v2} -log {compoundOutputFileLuid1} -qcResult {compoundOutputFileLuid2}
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation sets the next step to Advance and the samples to complete the protocol.

```
nextStep = ::ADVANCE::
```

Register Analysis Completed & Register Step Completed Automation

Automatically triggered on exit of the step, this automation performs the following actions:

- Registers the NextSeq 1000/2000 analysis status and result.
- Registers the completion time of the step.

These actions are done by publishing messages to CLPA through the LINK service. This automation is only used for CLPA support.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_
completed.groovy' && /opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/unified-product-analytics/automation/unified-
product-analytics-automation.jar script:executeUPAAutomationScript -i
{stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s
'com/illumina/upa/scripts/nextseq1k2k/nextseq1k2k_analysisrun_completed.groovy'
```

Master Step Fields

The following table lists the master step fields that are configured on the Demultiplexing (NextSeq 1000/2000 Sequencing v2.4) master step.

Table 311 Master Step Field Configuration for Demultiplexing (NextSeq 1000/2000 Sequencing v2.4) Step

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Criteria 1 - Source Data Field	Text Dropdown	Custom Entries	Presets • # Reads • # Perfect Index Reads • # One Mismatch Index Reads
Criteria 2 - Source Data Field	Text Dropdown	Custom Entries	Presets • # Reads • # Perfect Index Reads • # One Mismatch Index Reads
Criteria 1 - Operator	Text Dropdown	Custom Entries	Presets • >= • <= • = • !=

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Criteria 2 - Operator	Text Dropdown	Custom Entries	Presets • >= • <= • = • !=
Criteria 1 - Threshold Value	Numeric		Valid integer value
Criteria 2 - Threshold Value	Numeric		Valid integer value
Log	Multiline Text	Read Only	
Analysis Completed Time	Text	Read Only	Hidden Do not delete, rename, or modify. These actions will break the integration.
Analysis Started Time	Text	Read Only	Hidden Do not delete, rename, or modify. These actions will break the integration.
Analysis Status	Text	Read Only	
BSSH Analysis ID	Text	Read Only	Hidden Do not delete, rename, or modify. These actions will break the integration.

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Reagent Cartridge ID	Text	Read Only	Hidden Used for parsing demultiplexing results Do not delete, rename, or modify. These actions will break the integration.

Global Fields

The following table lists the global fields that are configured on the Demultiplexing (NextSeq 1000/2000 Sequencing v2.4) step. These fields are used to display the demultiplexing result metrics for individual library in the sample pool.

The demultiplexing statistics are aggregated for multi-lane flow cells. For each demultiplexing field (for example, # Reads) displayed in the Sample Details table for the multi-lane flow cell (for example, P3), the displayed value is the sum of the statistics across all lanes.

Table 312 Global Field Configuration for Demultiplexing (NextSeq 1000/2000 Sequencing v2.4) Step

Field Name	Field Type	Field Constraints/Options
Index Sequence	Text	Read Only
# One Mismatch Index Reads	Numeric	Read Only
# Perfect Index Reads	Numeric	Read Only
# Reads	Numeric	Read Only

User Interaction

Refer to *NextSeq 1000/2000 Integration v2.5 Validation and Troubleshooting* on page 1461 for details.

How the NextSeq 1000/2000 Integration Works

Sample Sheet Generation and Planned Run Creation

On the Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.4) step, when the Validate Run Setup and Create Planned Run automation is triggered, the run and analysis parameters entered in the Run Details screen are used to create a planned run. Then, the run and analysis configuration is validated. If the validation fails, an error message is sent to Clarity LIMS. If the validation passes, the sample sheet is generated (and sent back to Clarity LIMS) and/or a planned run is created depending on the Run Mode selected.

- Local run mode: Only the sample sheet is generated and stored on Clarity LIMS. The sample sheet contains all the run and analysis configuration required to start the run on the instrument.
- Hybrid/Cloud run mode: A planned run is created. This planned run contains all the run and analysis configuration required to start the run on the instrument. The sample sheet is also generated and stored on Clarity LIMS for reference purposes.

For details on how to start a run using different run modes, refer to *Start A Sequencing Run On Instrument With Different Run Modes* on page 1459.

If a planned run with the same sample and project name has been created previously, the original case with the previous sample name can be reflected in the sample sheet generated from the Validate Run Setup and Create Planned Run automation. To resolve this issue, change the sample or project name in Clarity LIMS and run the automation again.

Run Status, Primary Metrics, and Analysis Results Parsing and Recording

After the sequencing run is started on the instrument, NextSeq 1000/2000 Control Software (NCS) notifies BaseSpace Sequence Hub that the sequencing run has started.

BaseSpace Sequence Hub then does the following actions:

- Local run mode: Creates a run on the BaseSpace Sequence Hub database with the run configuration information received from NCS.
- Hybrid/Cloud run mode: Creates a run on the BaseSpace Sequence Hub database by copying the run configuration information from the planned run using a special Cloud Run ID received from NCS.

After the run is created on BaseSpace Sequence Hub, a run update event message that carries the RunStarted equivalent message is created on BaseSpace Sequence Hub and delivered to Clarity LIMS SQS queue service. The message is captured and processed by the integration.

Based on the reagent cartridge barcode information in the event message (refer to the following example), the integration service looks in the queue of AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.4) step for a container with the same reagent cartridge barcode. When identified, the step is started automatically and the Run Status field on Record Details screen is updated to RunStarted. The integration service then updates all the other step fields (for example, Current Read, Output Folder) by parsing the details from the `RunParameters.xml` uploaded to BaseSpace Sequence Hub by NCS.

```
#Example of an run update event
{
"acl": [
"wid:61dc14e8-d823-3bd1-bea3-66f9c8388254"
],
"apiUrl":
"https://api.basespace.illumina.com/v2/runs/r.ZvTfDXsNhUyh2kA5S4Zewg",
"dateModified": "2020-03-26T09:19:58.5546497Z",
"flowcellBarcode": "11M11COMC",
"gdsFolderPath": "/Runs/200326_MP2-15_87_11M11COMC_r.ZvTfDXsNhUyh2kA5S4Zewg",
"gdsVolumeName": "bssh.61dc14e8d8233bd1bea366f9c8388254",
"id": "r.ZvTfDXsNhUyh2kA5S4Zewg",
"instrumentRunId": "200326_MP2-15_87_11M11COMC",
"name": "200326_MP2-15_87_11M11COMC",
"reagentBarcode": "EC0001990-EC5",
"sampleSheetName": "SampleSheet.csv",
"status": "Running",
"v1pre3Id": "194424236"
}
```


How the Integration Service Handles Run Status

The NCS continues to notify BaseSpace Sequence Hub of any run status changes throughout the course of the sequencing run. The run update event delivery process continues as described in the previous section.

The following table lists the run status details and how the integration service handles each status.

Run Status	Shown in Step	Details
RunStarted	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.4)	Run Status field is updated to RunStarted. All other step fields (except Run End Time) are updated based on `RunParameters.xml` detail obtained from BaseSpace Sequence Hub, the SQS event message, and the step field value from the Load to Reagent Cartridge (NextSeq 1000/2000 v2.4) step. Reagent cartridge lot information received from BaseSpace Sequence Hub is compared against the details of the pre-accessioned reagent cartridge on Clarity LIMS. • If the expiration date is different, it is updated to the date received from BaseSpace Sequence Hub. • If reagent cartridge is not found, it is created and set to Active. • If reagent cartridge is not in Active status, it is set to Active. Multiline Sequencing Log field is updated.

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Run Status	Shown in Step	Details
RunCompletedSuccessfully	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.4)	Run Status field is updated to RunCompletedSuccessfully Current Read and Current Cycle fields are updated to the final cycle number and read number based on `RunParameters.xml` detail obtained from BaseSpace Sequence Hub. Run End Time field is updated based on RunParameters.xml detail obtained from BaseSpace Sequence Hub. Sequencing run result (primary metrics) is downloaded from BaseSpace Sequence Hub and the fields in Sample Details table (eg, % Error Rate R1, % Phasing, etc.) are updated with the values. Multiline Sequencing Log field is updated. The integration automatically completes the step and routes the samples in the reagent cartridge container to the Demultiplexing (NextSeq 1000/2000 Sequencing v2.4) step.

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Run Status	Shown in Step	Details
RunErroredOut	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.4)	Run Status field is updated to RunErroredOut. Current Read and Current Cycle fields are updated to the final cycle number and read number that the run stopped based on `RunParameters.xml` detail obtained from BaseSpace Sequence Hub. Run End Time field is updated based on `RunParameters.xml` detail obtained from BaseSpace Sequence Hub. Sequencing run result (primary metrics) is downloaded from BaseSpace Sequence Hub and the fields in Sample Details table (eg, % Error Rate R1, % Phasing, etc.) are updated with the values. Multiline Sequencing Log field is updated. The integration does not automatically complete the step. Complete the step manually.

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Run Status	Shown in Step	Details
RunAbortedByUser	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.4)	Run Status field is updated to RunAbortedByUser. Current Read and Current Cycle fields are updated to the final cycle number and read number that was aborted based on `RunParameters.xml` details obtained from BaseSpace Sequence Hub. Run End Time field is updated based on RunParameters.xml detail obtained from BaseSpace Sequence Hub. Sequencing run result (primary metrics) is downloaded from BaseSpace Sequence Hub and the fields in Sample Details table (eg, % Error Rate R1, % Phasing, etc.) are updated with the values. Multiline Sequencing Log field is updated. The integration does not automatically complete the step. Complete the step manually.
AnalysisStarted	Demultiplexing (NextSeq 1000/2000 Sequencing v2.4)	The integration starts the step automatically for the reagent cartridge container. Multiline Sequencing Log field is updated. Analysis Status is set to Started.

Run Status	Shown in Step	Details
AnalysisCompleted	Demultiplexing (NextSeq 1000/2000 Sequencing v2.4)	The integration downloads the following metric files: • `Adapter_Metrics.csv` • `Demultiplex_stats.csv` • `Index_Hopping_Counts.csv` • `Quality_Metrics.csv` • `Top_Unknown_Barcodes.csv` The metric files are placed in the `Run_Metrics.zip` folder that is attached to the file placeholder in the step. The `Demultiplex_stats.csv` file contains the demultiplexing results from BaseSpace Sequence Hub. These results are parsed and the details are recorded in the Sample Details table for each library in the library pool. For multi-lane flow cells, the demultiplexing stats are aggregated for each stat across all lanes. Hidden master step fields are updated with information from BaseSpace Sequence Hub. The Analysis Status field is updated to Complete. The Multiline Sequencing Log field is updated. The integration does not automatically complete the step. Assign the QC flag to the individual library and manually complete the step.
AnalysisFailed	Demultiplexing (NextSeq 1000/2000 Sequencing v2.4)	Hidden step fields are updated with information from BaseSpace Sequence Hub. The Analysis Status field is updated to either TimedOut or Aborted. The Multiline Sequencing Log field is updated. The integration does not automatically complete the step. Assign the QC flag to the individual library and manually complete the step.

Start A Sequencing Run On Instrument With Different Run Modes

Refer to the *NextSeq 1000/2000 Product Documentation* for the following information:

- Configuring a run with the Proactive, Run Monitory and Storage option. Local runs are done in Local mode, and cloud or hybrid runs are done in Cloud or Hybrid mode.
- Starting a local, cloud, or hybrid run.

Requeue Samples for Cloud Run

Clarity LIMS requires unique library names when samples are re-queued to the workflow in the NextSeq 1000/2000 integration. Assign unique names to libraries after going through library preparation.

The following steps are used to configure the library preparation workflow correctly before routing the samples for re-queuing:

1. From Configuration, select the Lab Work tab.
2. Search for the library preparation workflow used for the re-queued samples.
3. In the Master Step of the library preparation workflow, modify the naming convention under Step Type to generate unique library names (for example, appending LIMS ID to the default naming convention, like OutputItemLIMSID).
4. Select Save.

Components Installed

The following sections describe the various components that are installed by default as part of this integration. These components include the following items:

- Reagent categories/label groups
- Reagent kits
- Control types
- Containers

Information on installed workflows, protocols, steps, and automation points is provided in the Workflows, Protocols, and Steps section of *NextSeq 1000/2000 Integration v2.5 Validation and Troubleshooting* on page 1461.

Reagent Categories/Label Groups

- TruSeq HT adapters v2 (D7-D5)

Reagent Kits

- Resuspension Buffer (RSB)
- NextSeq 1000/2000 reagent cartridge

Container Types

- Tube
- 96-well plate
- NextSeq 1000/2000 reagent cartridge

Control Types

- PhiX v3

This integration supports the NextSeq 1000/2000 reagent cartridge with barcode provided in the format [A-Z]{2}[0-9]{7}-[A-Z0-9]{4} (for example, EC1234567-EC03).

Rules and Constraints

> The NextSeq 1000/2000 Reagent Cartridge barcode should not be modified after a successful validation. Modifications can cause issues when Clarity LIMS tries to update the status and sample details of subsequent steps.

- The workflow configuration contains several validation checks. To make sure that the calculations work properly, it is important that you do not disable any of this validation logic. The validation checks determine:
  - Which samples, and how many, can enter each step together.
  - Which samples, and how many, can be pooled together.
- The NextSeq 1000/2000 reagent cartridge barcode must be unique. There should not be multiple NextSeq 1000/2000 reagent cartridge containers in the system with the same name.
- Reagent labels (indexes) must be unique.
- One library pool can only contain one library or control with no label (index).
- Do not manually start or complete the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.4) step. This step is a fully automated step, and the integration service does not update samples correctly if they have been manually started.
- Do not manually start the Demultiplexing (NextSeq 1000/2000 Sequencing v2.4) step. This step is semi-automated, and the integration service does not update the demultiplexing result correctly if they have been manually started.
- For the automated run to start successfully, select Validate Run Setup and Create Planned Run in the Load to Reagent Cartridge step.

NextSeq 1000/2000 Integration v2.5 Validation and Troubleshooting

This section explains how to validate the installation of the Illumina NextSeq 1000/2000 Integration Package v2.5.

The validation process involves the following items:

- Running samples through the Library Prep Validation v2.3.3 workflow.
  - The workflow contains a single-step protocol that models the library prep workflow required to produce libraries tagged with index sequences. At the end of the step, these libraries are routed to NextSeq 1000/2000 Sequencing v2.4 workflow.
- Running the libraries through the NextSeq 1000/2000 Sequencing v2.4 workflow. This process validates the following information:
  - Successful sequential step advancement of samples in the following steps:
    1. Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.4)
    2. Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.4)
    3. AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.4)
    4. Demultiplexing (NextSeq 1000/2000 Sequencing v2.4)
  - Automatic validation of run setup information before sample sheet generation or planned run creation.
  - Automated generation of a sample sheet. The sample sheet is used to start the sequencing run on NextSeq 1000/2000 Control Software (NCS) via Local run mode.
  - Automated creation of a planned run on Illumina Connected Analytics. The NextSeq 1000/2000 Control Software retrieves the planned run to start the run via Hybrid or Cloud run mode.
  - Automated tracking of the NextSeq 1000/2000 sequencing run and parsing of run statistics from BaseSpace Sequence Hub into Clarity LIMS using the integration service.
  - Automated tracking of the secondary analysis using DRAGEN (up to BCL Convert only).
  - Parsing of demultiplexing metrics from BaseSpace Sequence Hub into Clarity LIMS using the integration service.

Before running the validation steps below, these steps assume that the NextSeq 1000/2000 Integration Package v2.5 is installed, and that you have imported the default Clarity LIMS configuration.

For information on how the integration works, refer to *NextSeq 1000/2000 Integration v2.5 Configuration and User Interaction* on page 1425.

Activate Workflow, Create Project, Add and Assign Samples

The following steps set up Clarity LIMS in preparation for running samples through the Library Prep Validation v2.3.3 and NextSeq 1000/2000 Sequencing v2.4 workflows.

1. On the Configuration tab, under Workflows, activate both the Library Prep Validation v2.3.3 and NextSeq 1000/2000 Sequencing v2.4 workflows.
2. On the Projects and Samples screen, create a project and add samples to it.

   Use only alphanumeric, dash, and underscore characters in the submitted sample names. Failing to do so causes sample sheet validation failure in Load To Reagent Cartridge step.

3. Assign the samples to the Library Prep Validation v2.3.3 workflow.

Library Prep Protocol: Library Prep Validation v2.3.3

This single-step protocol models the library prep required to produce libraries tagged with index sequences that are ready for the NextSeq 1000/2000 Sequencing v2.4 workflow.

Run Library Prep Validation v2.3.3 Step

1. In Lab View, locate the Library Prep Validation v2.3.3 protocol.
   Samples are queued for the Library Prep Validation v2.3.3 step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select the label groups to use.
   - TruSeq HT Adapters v2 (D7-D5) is the default label group delivered by Library Prep Validation v2.3.3 protocol.
   - For other reagent labels (indexes), manually create them in the Configuration screen under Consumables > Labels.

     For more information on how to create reagent label group, refer to the *Add and Configure Labels and Label Groups* section in the Clarity LIMS Core Functionality documentation.
4. Select Begin Work.
5. On the Placement screen, perform the following actions:
   a. In the Placed Samples area in the Container field, scan or enter the barcode of the destination container.
   b. Select the samples from the Samples to be Placed area, then drag them over to the container wells on the right.
   c. Select Add Labels.
6. On the Add Labels screen, the Labels to Add list is populated with labels from the TruSeq HT Adapters v2 (D7-D5) label group. This label group is the only one configured in the out-of-the-box integration. It is used by default in this step.
   a. Select labels on the left, and drag them over to the container on the right to assign them to placed samples.
   b. Select Record Details.
7. On the Record Details screen, perform the following actions:
   a. In the Sample Details table, enter values for Molarity (nM) for all the individual sample. Enter values individually or use the copy/paste functionality to enter multiple values from a spreadsheet.
      - Molarity (nM) values are used in Calculate Volume automation script in Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.4) step.

> If Molarity (nM) values are not entered, the Calculate Volume automation script does not work properly.

b. The Sequencing Instrument column indicates the instrument to be used for the run. Select NextSeq 1000/2000.

c. Populate the fields in this column using one of the following methods:

- To populate fields individually for each sample, select from the drop-down list in each row.
- To populate fields all at one time, use the Copy and Paste buttons to enter multiple values from a spreadsheet (refer to the following note).

Alternatively, select inside the table and press Ctrl + A to select all rows. At the top of the table, select Sequencing Instrument, then select NextSeq 1000/2000 from the adjacent drop-down list. Select Apply.

> If working with many samples, copy and paste to insert multiple values from a spreadsheet as follows.

a. Select the Copy button to copy the sample information from Clarity LIMS to the clipboard.

b. Open a blank Excel document and paste the contents of the clipboard into the spreadsheet.

c. In the spreadsheet, enter the Molarity (nM) and Sequencing Instrument values. Select the entire spreadsheet, including the headers, (Ctrl + A) and copy (Ctrl + C) the information to the clipboard.

d. In Clarity LIMS, select the Paste button. Paste the contents of the clipboard (Ctrl + V) into the Update Table window and then select Update to paste into the Sample Details table.

8. Select Next Steps.
   This selection triggers the Next Steps automation, which sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step successfully.
9. On the Assign Next Steps screen in the Samples table, the Next Step for all samples is prepopulated with Remove from workflow.

> Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.

10. Select Finish Step.
    On exit from the step, the Routing Script automation is triggered. This automation assigns samples to the first step of the NextSeq 1000/2000 Sequencing v2.4 workflow, which is Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.4) step.

NextSeq 1000/2000 Sequencing v2.4 Protocol

Step 1: Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.4)

1. In Lab View, locate the NextSeq 1000/2000 Sequencing v2.4 protocol. The samples are queued for the Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.4) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Pooling screen, perform the following actions:
   a. Create a pool by dragging samples into the Pool Creator.

      Do not create more than one pool for this step. The Calculate Volumes automation in this step supports one pool only.

   b. Type a name for the pool or accept the default name (Pool #1).
   c. Select Record Details.
      On the Record Details screen, the Reagent Lot Tracking section tracks the Resuspension Buffer (RSB) lot information used in the step. To add and activate additional reagent lots, refer to *Add and Configure Reagent Kits and Lots* in the Clarity LIMS Core Functionality documentation.
5. In the Reagent Lot Tracking section, select from the active lots displayed in the drop-down list.
   The Step Details area contains two required fields:
   - Final Loading Concentration (pM)—The value entered in this field is the recommended final loading concentration specified in the *NextSeq 1000/2000 Product Documentation*. The value depends on the library type. The default drop-down list contains the values 650, 750, 1000, and 2000. A custom value is acceptable.
   - Final Loading Volume (µl)—The value in this field is the final loading volume of the pool into the reagent cartridge. The field is prepopulated with the configured default value, 24 µl, specified in the *NextSeq 1000/2000 Product Documentation*. The value is editable when more volume is necessary.
6. Select Calculate Volumes.
   This selection triggers the Calculate Volumes automation. This automation calculates the volume required for each library to form a pool that has the concentration and volume specified in the step details fields.
   The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains volume information of each of the samples and RSB buffer to add to the pool. Select the file to download it, then open it in Excel.
7. In the Sample Details table, select the pool icon to view details on the pool composition.
8. Select Next Steps.
   This selection triggers the Set Next Step automation, which sets the next step for samples to ADVANCE, advancing them to the next step in the protocol. The next step is Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.4).

On the Assign Next Steps screen, the next step for samples is already set to the next step in the workflow. The next step is Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.4).

9. Select Finish Step.

At the end of this step, the pool of samples automatically advances to Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.4) step.

Step 2: Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.4)

1. In Lab View, locate the NextSeq 1000/2000 Sequencing v2.4 protocol. The pool of samples is queued for the Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.4) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
   The Validate Single Input automation is triggered. This automation checks that there is only one container input to the step.
4. On the Placement screen, perform the following actions:
   a. Drag the pool into the NextSeq 1000/2000 Reagent Cartridge field in the Placed Samples area.
   b. Scan or type the barcode of the reagent cartridge into the NextSeq 1000/2000 Reagent Cartridge field.
   c. Select Record Details.
   On exit of the Placement screen, the Validate Reagent Cartridge Barcode automation checks that the reagent cartridge barcode conforms to the barcode mask [A-Z]{2}[0-9]{7}-[A-Z0-9]{4}. If not, an error message displays.

   > The NextSeq 1000/2000 Reagent Cartridge barcode should not be modified after a successful validation. Modifications can cause issues when Clarity LIMS tries to update the status and sample details of subsequent steps.

   On entry to the Record Details screen, the Retrieve Analysis Workflow Versions automation fetches the available analysis workflow versions from ICA. It then updates the preset values of both Local Analysis Workflow Versions & Cloud Analysis Workflow Versions field.
5. On the Record Details screen, the Reagent Lot Tracking section tracks the NextSeq 1000/2000 Reagent Cartridge lot information used in the step. Follow the steps in Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.4) step if you must add a lot.
6. In the Reagent Lot Tracking section, select from the active lots displayed in the drop-down list.
7. The fields displayed on the Record Details screen are used to create planned run and generate the sample sheet file.
   - Run Name—Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.
   - Instrument Type—Select from preset options (NextSeq 1000 or NextSeq 2000).

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- Run Mode—Select from preset options: Local, Hybrid, Cloud. For more information on the different run modes, refer to *Run Mode Details* on page 1468.
- Paired End—Select from preset options (True or False).
- Read 1 Cycles—Select from preset options (301, 151, 101, 51) or type a custom value.
- Read 2 Cycles—Select from preset options (301, 151, 101, 51) or type a custom value.
- Index Reads—Select from preset options (No Index, Single Index, Dual Index).
- Index Read 1—Select from preset options (0, 6, 8) or type a custom value.
- Index Read 2—Select from preset options (0, 6, 8) or type a custom value.
- Analysis Workflow—Read only. This field is set to GenerateFASTQ.
- [Optional] Local Analysis Workflow Versions—Default is None. Only mandatory when Run Mode = Local/Hybrid and Analysis Workflow = GenerateFASTQ. Select according to the DRAGEN version installed on the instrument.
- [Optional] Cloud Analysis Workflow Versions—Default is None. Only mandatory when Run Mode = Cloud and Analysis Workflow = GenerateFASTQ. Select the cloud DRAGEN version to be used.
- FASTQ Compression Format:
  - If Run Mode = Local/Hybrid, Analysis Workflow = GenerateFASTQ, and Local Analysis Workflow Version >= 3.7.4, select gzip or DRAGEN.
  - If Run Mode = Cloud, Analysis Workflow = GenerateFASTQ, and Local Analysis Workflow Version >= 3.7.4, field is set to gzip automatically.
  - Otherwise, select None.
- [Optional] Adapter Sequence Read 1—Enter the Read 1 adapter sequence of the index adapter kit.
- [Optional] Adapter Sequence Read 2—Enter the Read 2 adapter sequence of the index adapter kit.
- [Optional] Barcode Mismatches Index 1—Select from preset options (0, 1, 2). Leave it blank if you are unsure.
- [Optional] Barcode Mismatches Index 2—Select from preset options (0, 1, 2). Leave it blank if you are unsure.
- Override Cycles—[Optional] String used to specify UMI cycles and mask out cycles of a read (for example, N1Y150;I8;I7N1;Y141U10). Leave it blank if you are unsure.
- Instructions—Read only.

8. On the Record Details screen, select Validate Run Setup and Create Planned Run.
   This selection triggers the automation script, which does the following actions:
   - Validates the parameters entered on the Record Details screen.

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- If Run Mode is Hybrid or Cloud, create the planned run.
- Generates the sample sheet and attaches it to the placeholder in the Files area of the Record Details screen for all Run Mode types.

9. Select Next Steps.
   On the Assign Next Steps screen, the next step for samples is set to the next step in the workflow. The next step is AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.4).
10. Select Finish Step.

At the end of this step, the pool of samples automatically advances to and is queued for the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3) step. The sequencing run is ready to be started. For details on how to start the sequencing run for different run modes, refer to *NextSeq 1000/2000 Integration v2.5 Configuration and User Interaction* on page 1425.

Run Mode Details

The run mode applies to all runs and determines how the run and analysis parameters are configured and how to analyze data.

- Local Mode—NextSeq 1000/2000 Integration service generates the sample sheet. Run/Analysis configuration is imported manually into NextSeq 1000/2000 instrument via a sample sheet to start the run. The downstream secondary analysis is done using the onboard DRAGEN module.
- Hybrid Mode—The planned run is created in BaseSpace Sequence Hub based on the run/analysis data from the NextSeq 1000/2000 Integration service. Run/Analysis configuration is downloaded to NextSeq 1000/2000 instrument to start the run. The downstream secondary analysis is done using the onboard DRAGEN module.
- Cloud Mode—The planned run is created in BaseSpace Sequence Hub based on the run/analysis data from the NextSeq 1000/2000 Integration service. Run/Analysis configuration is downloaded to NextSeq 1000/2000 instrument to start the run. The downstream secondary analysis is performed in the cloud.

Step 3: AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.4)

This step is fully automated.

> Do not add samples to the Ice Bucket or start or complete the step. The integration completes these actions automatically.

The integration starts the step automatically and data from the run is parsed back to Clarity LIMS. No user interaction is required. However, you can open and review the various stages of the step in Clarity LIMS. Do not perform any action when reviewing the data.

Read summary metrics are recorded for the library pool in the Step Details section and the Sample Details table.

Values are populated in the following master step fields:

- Run Name
- Current Read
- Flow Cell ID
- Reagent Cartridge Lot Number
- Instrument ID
- Run Status
- Current Cycle
- Flow Cell Lot Number
- Instrument Platform
- Instrument Control Software Version
- Output Folder
- Secondary Analysis Workflow
- Reagent Cartridge ID
- Instrument Type
- RTA Version
- Sequencing Log

The summary metrics (per run level) populate in the following global fields.

- % Bases >=Q30 R1
- % Bases >=Q30 R2
- % Error Rate R1
- % Error Rate R2
- Yield (Gb) R1
- Yield (Gb) R2
- Reads PF R1
- Reads PF R2
- %PF R1
- %PF R2
- % Aligned R1
- % Aligned R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1

- % Prephasing R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- Cluster Density R1
- Cluster Density R2

At the end of this step, the pool of samples automatically advances to (and queues for) the Demultiplexing (NextSeq 1000/2000 Sequencing v2.4) step.

Step 4: Demultiplexing (NextSeq 1000/2000 Sequencing v2.4)

This step is semi-automated.

> Do not add samples to the Ice Bucket or start or complete the step. The integration completes these actions automatically.

The integration starts the step automatically and demultiplexing data from the GenerateFASTQ secondary analysis is parsed back to Clarity LIMS. Review the data parsed and assign QC, depending on the criteria set, and complete the step.

In the Record Details screen, perform the following actions:

1. Review demultiplexing data.
   - Demultiplexing metrics are recorded for the library pool in the Sample Details table. Metrics include columns for # Reads, # Perfect Index Reads, and # One Mismatch Index Reads.
   - The following metric files generated by the DRAGEN onboard or Cloud analysis module are stored under the Files section as `Run_Metrics.zip`:
     - `Adapter_Metrics.csv`
     - `Demultiplex_Stats.csv`
     - `Index_Hopping_Counts.csv`
     - `Quality_Metrics.csv`
     - `Top_Unknown_Barcodes.csv`
   - The Analysis Status step field is updated with the analysis status.
2. Assign QC flags to all the individual sample. There are two ways of doing this step.
   a. Manually assign QC flags through the QC column in Sample Details table.
   b. Automatically assign QC flag by running Assign QC flags automation. This option is for scenarios where a huge number of libraries are involved.

   In the Step Details section, the following step fields are visible. (N is the number of criteria. You can use one or more criteria.)
   - Criteria N - Source Data Field—Select from preset options (for example, # Reads).

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- Criteria N - Operator—Select from preset options (for example, >= (greater than or equal to)).
- Criteria N - Threshold Value—Enter the desired threshold value.

3. After filling up the criteria fields, select Assign QC flags. This selection triggers the automation script, which loops through each library in the pool and apply QC flag base on the criteria set previously.
   This automation also generates an AssignQC Result file under Files section.
4. Select Next Steps.
   On the Assign Next Steps screen, the Next Step field for all samples is prepopulated with Mark protocol as complete.
5. Select Finish Step.

At this point, the whole NextSeq 1000/2000 Integration workflow is fully validated.

Troubleshooting

If an automation trigger does not appear to run its corresponding scripts, refer to *Troubleshooting Automation* in the Clarity LIMS API documentation.

If the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.4) step starts but does not finish, complete the following steps:

1. Log in to Clarity LIMS using the default user account and use one of the following methods to open the step in Clarity LIMS:
   - Method 1: In Lab View, find the step in the Recent Activities pane.
   - Method 2: Search for the step in Clarity LIMS using reagent cartridge barcode as the search term.
2. On the Record Details screen, locate the Sequencing Log field.
   The multiline text field contains logging information.

If you cannot reach the Record Details screen, or if the Sequencing Log field does not contain enough information to resolve the issue, contact the Clarity LIMS support team. Supply the relevant information from the troubleshooting steps already performed.

NextSeq 1000/2000 Integration v2.5 Manual Upgrade

NextSeq 1000/2000 Integration v2.5 supports future ICA services and includes new custom fields and modifications to existing automations. The integration package requires the NextSeq 1000/2000 Sequencing v2.4 workflow. You can install this workflow through the Illumina Preset Protocols (IPP). If you do not want to upgrade through the IPP, you can manually upgrade the workflow configuration.

Modify the Existing NextSeq 1000/2000 Workflow

To modify the existing NextSeq 1000/2000 Sequencing v2.3 workflow, you must make the following changes:

- Add the Measurement global field.
- Modify the Master Step field.
- Modify the Demultiplexing (NextSeq 1000/2000 Sequencing v2.3) master and protocol steps.
- Update the Validate Reagent Cartridge Barcode step automation.

Add Index Sequence Global Field

1. From Configuration, select the Custom Fields tab.
2. In Global Fields, create a Measurement field with the following properties:
   - Field Name—Index Sequencing
   - Field Type—Text
   - Required Field—No
   - Read Only—Yes
3. Select Save.

Modify Master Step Field

1. From Configuration, select the Custom Fields tab.
2. Select the Master Step Fields tab.
3. Select Demultiplexing (NextSeq 1000/2000 Sequencing v2.3) to display the applicable fields.
4. Select Criteria 1 - Source Data Field.
5. In the Additional Options pane, remove # of > = Q30 Bases (PF) from the Dropdown Items list.
6. Select Criteria 2 - Source Data Field.
7. In the Additional Options pane, remove # of > = Q30 Bases (PF) from the Dropdown Items list.
8. Select Save.

Modify Demultiplexing (NextSeq 1000/2000 Sequencing v2.3) Master Step

1. From Configuration, select the Lab Work tab.
2. Navigate to the Steps pane and select Demultiplexing (NextSeq 1000/2000 Sequencing v2.3).
3. Select the Master Step tab, and then select Record Details.
4. In the Step File Placeholders pane, rename Demultiplex Stats to Run Metrics.
5. Select Save.

Modify Demultiplexing (NextSeq 1000/2000 Sequencing v2.3) Protocol Step

1. From Configuration, select the Lab Work tab.
2. Navigate to the Steps pane and select Demultiplexing (NextSeq 1000/2000 Sequencing v2.3).

3. Select the Step tab, and then select Record Details.
4. Add the Analysis Status field as follows.
   a. In the Step Data pane, select + to open the Master Step Fields pane.
   b. Select Analysis Status, and then select the checkmark to add the field to the Master Step Fields list.
5. Update the Sample Table as follows.
   a. In the Sample Table pane, navigate to the Table Columns pane and remove the following fields:
      - [Measurement] # of > = Q30 Bases (PF)
      - [Measurement] Mean Quality Score (PF)
   b. Select + to open the Fields pane.
   c. Select Measurement to open the field drop-down list.
   d. Select Index Sequence.
   e. Reorder the table columns as follows.
      - [Derived Sample] Sample Name
      - [Measurement] Index Sequence
      - [Container] Well
      - [Measurement] # Reads
      - [Measurement] # Perfect Index Reads
      - [Measurement] # One Mismatch Index Reads
      - [Container] Container Name
      - [Project] Project Name
   f. Select Save.

Update Validate Reagent Cartridge Barcode Step Automation

1. From Configuration, select the Automation tab, and then select the Step Automation tab.
2. Select Validate Reagent Cartridge Barcode.
3. In Automation Details, replace the content of the Command Line field with the following command:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -exp 'if (!output.container.name.matches( ::[A-Z]{2}[0-9]{7}-[A-Z0-9]{4}:: ) ) {fail ( ::Invalid Reagent Cartridge Barcode. Please verify and try again.:: ) }' -log {compoundOutputFileLuid1}"
```

4. Select Save.

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Modify NextSeq 1000/2000 Sequencing v2.5 Integration Package Configuration

When the `/opt/gls/clarity/config/configure_nextseq1k2k.sh` script is running as part of the installation and configuration process, do not use the default names for the automated sequencing and demultiplexing steps. Instead, use the names in the modified workflow.

This integration moves the integration service and integration-related properties from the configuration files to the database.

To make modifications after configuration, use the omxProps-ConfigTool to update the following properties:

Property	Description
integration.nextseq1k2k.v2.automatedStepNames	Comma-separated step names for the automated sequencing step
integration.nextseq1k2k.v2.analysisStepNames	Comma-separated step names for the demultiplexing step

NextSeq 1000/2000 Integration v2.5 Release Notes

These Release Notes describe the key changes to software components for the Clarity LIMS NextSeq 1000/2000 Integration Package v2.5.

Compatibility

This version of the integration package is compatible with the following software:

- Clarity LIMS v6.2 and later
- NextSeq 1000/2000 Instrument Control Software (NCS) v1.5.0

New Features

- Updates the Demultiplexing step in NextSeq 1000/2000 Sequencing workflow with the following changes:
  - The Sample Details table displays the index sequence.
  - The Sample Details table only displays the following demultiplexing stats due to changes in `Demultiplex_Stats.csv`:
    - # Reads
    - # Perfect Index Reads
    - # One Mismatch Index Reads
  - The demultiplexing stats displayed are aggregated for multi-lane flow cells (for example, P3).
  - The following run metrics files are included in `Run_Metrics.zip` and attached to the step:
    - `Adapter_Metrics.csv`
    - `Demultiplex_Stats.csv`

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  - `Index_Hopping_Counts.csv`
  - `Quality_Metrics.csv`
  - `Top_Unknown_Barcodes.csv`
- Analysis Status is now visible on the step.

- The integration service and integration-related properties have been moved from configuration files to database.
- Supports DRAGEN BCL Convert v4.2.7 analysis, including:
  - Cloud BCL Convert v4.2.7 running under the ICA Workflow Session Tracking application
  - Local BCL Convert v4.2.7

Defect Repairs

- Demultiplexing stats are now correctly displayed in the Sample Details table in Demultiplexing step when the same samples with different indexes are in the run. For multi-lane flow cells, the demultiplexing stats are aggregated.
- The Validate Reagent Cartridge Barcode automation in the Load to Reagent Cartridge step now only accepts the [A-Z]{2}[0-9]{7}-[A-Z0-9]{4} barcode format.

Known Issues

- Sequencing run error triggered by disk space full on instrument causes `RunParameters.xml` to be empty and the run event cannot be processed on Clarity LIMS. This error is unlikely to occur as pre-run checks on the instrument check for sufficient disk space before run is started.

NextSeq 1000/2000 Integration v2.4 Configuration and User Interaction

The Illumina NextSeq 1000/2000 Integration Package v2.4 supports the integration of Clarity LIMS to Illumina NextSeq 1000/2000 sequencing systems.

For instructions on validating and troubleshooting the NextSeq 1000/2000 Integration, refer to *NextSeq 1000/2000 Integration v2.4 Validation and Troubleshooting* on page 1512.

> The configuration provided in this integration has been established to support NextSeq 1000/2000 lab processes. Any configuration changes to protocols or workflows—including renaming protocols, steps, and fields—could break the process.

Overview

The integration includes the following features:

- Preconfigured NextSeq 1000/2000 Sequencing v2.3 workflow that maps to lab protocols and instrument runs.
- The following preconfigured protocols:
  - Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3)

    - Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3)
    - AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3)
    - Demultiplexing (NextSeq 1000/2000 Sequencing v2.3)
- Automatic validation of run setup information before sample sheet generation or planned run creation.
- Automated generation of a sample sheet. The sample sheet is used to start the sequencing run on NextSeq 1000/2000 Control Software (NCS) via Local run mode.
- Automated creation of a planned run on Illumina Connected Analytics (ICA). The NCS retrieves and starts the planned run via Hybrid or Cloud run mode.
- Automated tracking of the NextSeq 1000/2000 sequencing run and parsing of run statistics from BaseSpace Sequence Hub into Clarity LIMS using SIS integration service.
- Automated tracking of the secondary analysis using DRAGEN (up to BCL Convert only) and parsing of demultiplexing metrics from BaseSpace Sequence Hub into Clarity LIMS using SIS integration service.
- Support for parsing the unicode `Parameter.xml` file generated by NCS v1.5.
- [Optional] Preconfigured Library Prep Validation v2.3.1 workflow used for validation purposes only. The workflow contains a single-step protocol that models the library prep workflow required to produce libraries tagged with index sequences. At the end of the step, these libraries are routed to NextSeq 1000/2000 Sequencing v2.3 workflow. For more information, refer to *NextSeq 1000/2000 Integration v2.4 Validation and Troubleshooting* on page 1512.
- [Optional] An integration of Clarity LIMS Product Analytics (CLPA) that make Clarity LIMS data available to the ICA and CLPA. This integration also requires installation and configuration. For more information, refer to *Clarity LIMS Product Analytics Integration v1.2 Configuration and User Interaction* on page 2399.

Prerequisites and Assumptions

The Illumina NextSeq 1000/2000 Integration Package v2.4 is compatible with Illumina cloud hosted deployments only.

It is assumed that samples entering the NextSeq 1000/2000 Sequencing v2.3 workflow have gone through library preparation and quantification processes. Before they are assigned to the workflow, samples have completed the following actions:

- Samples have been accessioned into Clarity LIMS.
- Samples have been run through QC and library prep.
- Samples have the Molarity (nM) global field set to some value.
  The Calculate Volumes automation in the Library Pooling and Dilution step requires a value in the Molarity (nM) global field.

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For more information on sample accessioning, refer to *Sample Accessioning* and *Upload and Modify Samples* in the Getting Started section of the Clarity LIMS Core Functionality documentation.

You can assign samples to workflows automatically, using a routing script, or manually—from the Projects & Samples dashboard. Refer to *Assign and Process Samples* in the Clarity LIMS Core Functionality documentation.

Workflows, Protocols, and Steps

The NextSeq 1000/2000 Integration Package v2.4 includes the following workflows:

- NextSeq 1000/2000 Sequencing v2.3
  Samples are routed to this protocol after they have gone through Library Prep Validation v2.3.1 (or any other library prep workflow). Samples are pooled, diluted to final loading concentration, and added to a reagent cartridge in preparation for the sequencing run.
- [Optional] Library Prep Validation v2.3.1 (recommended for validation purposes).

The workflow configuration includes a global field named Illumina Universal Sample Identifier. This text field is optional and reserved for a future feature. The field value is not required for this integration.The following table lists and describes the protocols and steps included in these workflows.

> The workflow configuration includes a global field named Illumina Universal Sample Identifier. This text field is reserved for Clarity LIMS Product Analytics support. The field is optional and not required for this integration.

Table 313 Illumina NextSeq 1000/2000 Integration Package v2.4 Protocols and Steps

Protocol Name	Protocol Purpose	Steps Included in Protocol
Library Prep Validation v2.3.1 Workflow		
Library Prep Validation v2.3.1	Included for validation purposes only, this protocol models the library prep steps required to advance samples to the NextSeq 1000/2000 Sequencing v2.3 workflow. The protocol contains a single step—Library Prep Validation v2.3.1. At the end of this step, a routing script sends the samples to the first step of the NextSeq 1000/2000 Sequencing v2.3 workflow. The first step is Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3).	1. Library Prep Validation v2.3.1

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Protocol Name	Protocol Purpose	Steps Included in Protocol
NextSeq 1000/2000 Sequencing v2.3 Workflow		

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Protocol Name	Protocol Purpose	Steps Included in Protocol
NextSeq 1000/2000 Sequencing v2.3	This protocol models the lab processes of starting a NextSeq 1000/2000 sequencing run. Details of the steps: 1. Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3) a. Samples are pooled and diluted to the final loading concentration with the help of the Calculate Volume script. 2. Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3) a. The library pool from step 1 is then ready to be loaded to the NextSeq 1000/2000 reagent cartridge. b. Run and analysis information is validated. c. Sample sheet is generated and/or planned run is created. 3. AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3) a. This step is a fully automated step that is started and completed automatically after the sequencing run is started and completed on the instrument side. b. All the metadata (eg, run configuration, primary run metrics, etc.) of the sequencing run are recorded automatically. Do not add samples to the Ice Bucket or start or complete the step. The integration completes these actions automatically. c. Generated data's secondary analysis is planned by default for the run. Samples continues	1. Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3) 2. Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3) 3. AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3) 4. Demultiplexing (NextSeq 1000/2000 Sequencing v2.3)

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Validation Workflow

The Library Prep Validation v2.3.1 workflow allows for validation of the system after installation is complete. For details, refer to *NextSeq 1000/2000 Integration v2.4 Validation and Troubleshooting* on page 1512.

Step Automations

Automation details are provided in the sections following the table.

For details on the validation workflow, refer to *NextSeq 1000/2000 Integration v2.4 Validation and Troubleshooting* on page 1512.

Table 314 NextSeq 1000/2000 Sequencing v2.3 Workflow Step Automations

Protocol	Step	Automation
NextSeq 1000/2000 Sequencing v2.3	Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3)	• Calculate Volumes • Register Pools & Register Step Completed[1] • Register Step Started[1] • Set Next Step
	Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3)	• Register Step Completed[1] • Retrieve Analysis Workflow Versions • Set Next Step • Validate Reagent Cartridge Barcode • Validate Run Setup and Create Planned Run • Validate Single Input & Register Step Started[2]
	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3)	• Register NextSeq1k2k RunCompleted & Register Step Completed[1] • Register Step Started & Register NextSeq1k2k Run & Register NextSeq1k2k Run Association & Register NextSeq1k2k RunStarted[1]
	Demultiplexing (NextSeq 1000/2000 Sequencing v2.3)	• Assign Demultiplex QC flags • Register Analysis Completed & Register Step Completed[1] • Register Step Started[1] • Set Next Step

[1] These automations are required for Clarity LIMS Product Analytics support only.
[2] These automations are required for the NextSeq 1000/2000 Sequencing v2.3 workflows and contain additional logic needed for Clarity LIMS Product Analytics support. If you would like to remove Clarity LIMS Product Analytics support, contact Illumina Support.

Step 1: Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3)

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In this step, the addition of RSB dilutes pooled samples. Manually create a working pool based on the final loading concentration required.

Create one pool per step. The Calculate Volumes automation supports one pool only.

Register Step Started

This automation registers the start time of the step by publishing messages to CLPA through the LINK service. This automation is automatically triggered on entry to the step.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_started.groovy'
```

Calculate Volumes Automation

This automation is triggered when you select Calculate Volumes on the Record Details screen. The automation completes the following actions:

- Sets RSB Volume for Pool (µl) field value to 24 for calculation purpose of the 2 nM intermediate library pool.
- Copies the Final Loading Concentration (pM) from step inputs to step outputs.

```
-exp 'step.::RSB Volume for Pool (µl):: = 24; output.::Final Loading Concentration (pM):: = step.::Final Loading Concentration (pM)::'
```

- Calculate per sample volume required for each library to make the 2 nM intermediate library pool (24ul in volume).

```
-exp 'input.::Per Sample Volume (µl):: = ( 48/ input.::Molarity (nM):: ); step.::RSB Volume for Pool (µl):: -= input.::Per Sample Volume (µl)::'
```

- Calculate the volume required of the 2 nM library pool to be diluted further to the Final Loading Concentration (pM) with the Final Loading Volume (µl).

```
-exp 'step.::Library Pool Volume (µl):: = (step.::Final Loading Concentration (pM):: * step.::Final Loading Volume (µl)::/2000); output.::RSB Volume (µl):: = step.::Final Loading Volume (µl):: - step.::Library Pool Volume (µl)::'
```

- Uses the `NextSeq1K2K_Pool1.csv`, `NextSeq1K2K_Pool2.csv`, and `NextSeq1K2K_Pool3.csv` template files to generate a single CSV file containing information about the pool and the samples it contains. The generated file is stored in the Calculation File placeholder, in the Files section, for download.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} \
    script:driver_file_generator \
```

```
      -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NextSeq1K2K_
      Pool1.csv \
      -o 1.csv \
   script:driver_file_generator \
      -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NextSeq1K2K_
      Pool2.csv \
      -o 2.csv \
   script:driver_file_generator \
      -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NextSeq1K2K_
      Pool3.csv \
      -o 3.csv \
&& cat 1.csv 2.csv 3.csv > {compoundOutputFileLuid0}.csv
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol. The next step is Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3):

```
nextStep = ::ADVANCE::
```

Register Pools & Register Step Completed

This automation is automatically triggered on exit of the step and performs the following tasks:

- Registers the pool and library information used by the Clarity LIMS workflow. Samples in the pool are assumed to have gone through library preparation.
- Registers the completion time of the step by publishing messages to CLPA through the LINK service.

This automation is only used for CLPA support.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/pooling.groovy' &&
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_
completed.groovy'
```

Master Step Fields

The following fields are defined on the Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3) step.

Table 315 Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3) Master Step Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Final Loading Concentration (pM)	Numeric Dropdown	Required Field Custom Entries	Presets • 650 • 750 • 1000 • 2000
Final Loading Volume (µl)	Numeric	Required Field	Default • 24
Library Pool Volume (µl)	Numeric	Read Only	Hidden ⚠ This field is used by the Calculate Volumes automation. Removing, renaming, or modifying the field will break the automation.

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
RSB Volume for Pool (µl)	Numeric	Read Only	Hidden ⚠ This field is used by the Calculate Volumes automation. Removing, renaming, or modifying the field will break the automation.

Global Fields

The following table lists the global fields that are configured to display on the Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3) step.

Table 316 Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3) Global Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Final Loading Concentration (pM)	Numeric Dropdown	Required Field Custom Entries	Decimal places displayed = 0 Presets • 225 • 400
RSB Volume (µl)	Numeric	Read Only	Decimal places displayed = 2

User Interaction

Refer to *NextSeq 1000/2000 Integration v2.4 Validation and Troubleshooting* on page 1512 for details.

Step 2: Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3)

In this step, scan the reagent cartridge barcode into Clarity LIMS, then manually place the working pool into the reagent cartridge for the NextSeq 1000/2000 run. This step validates the run setup and analysis information and generates the sample sheet file and/or creates a planned run on Illumina Connected Analytics, depending on the Run Mode selected.

The NextSeq 1000/2000 reagent cartridges support different read cycle numbers. Make sure that the read cycle values configured for the planned run are within the maximum allowable reads for the cartridge type.

Validate Single Input & Register Step Started Automation

Automatically triggered at the beginning of the step, this automation does the following actions:

- Checks that there is only one container input to the step. This script is required for the NextSeq 1000/2000 Sequencing v2.3 workflow.

```
script:validateSampleCount -min 1 -max 1
```

- Registers the start time of the step by publishing messages to Clarity LIMS Product Analytics through the LINK service. This script is only used for Clarity LIMS Product Analytics support.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_
started.groovy'
```

Validate Reagent Cartridge Barcode Automation

Automatically triggered on exit of the Placement screen, the following automation validates the reagent cartridge barcode to make sure it conforms to the barcode mask [A-Za-z]{2}[0-9]{7}-[A-Za-z0-9]{4}:

```
if (!output.container.name.matches( ::[A-Za-z]{2}[0-9]{7}-[A-Za-z0-9]{4}:: ) )
{fail ( ::Invalid Reagent Cartridge Barcode. Please verify and try again.:: ) }
```

Retrieve Analysis Workflow Versions Automation

Automatically triggered upon entry of the Record Details screen, this automation completes the following actions:

- Fetches the available analysis workflow versions from ICA.
- Updates the preset values of both Local Analysis Workflow Versions & Cloud Analysis Workflow Versions fields.

```
/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/nextseq1k2k-
automation.jar script:retrieve_analysis_workflow_versions -i {stepURI:v2} -u
{username} -p {password} -l {compoundOutputFileLuid2}
```

Do not modify or disable the Retrieve Analysis Workflow Versions automation script. Modifying or disabling the script breaks the integration.

Validate Run Setup and Create Planned Run Automation

Automatically triggered when a button on the Record Details screen is selected, this automation does the following actions:

- Validates the parameters entered on the Record Details screen. These parameters are used to set up the run and generate the sample sheet file and/or create the planned run on ICA.
  - Run Name can only contain alphanumeric, dash, underscore, or period characters. Spaces are not permitted.

```
if (!step.::Run Name::.matches(::[a-zA-Z0-9-_.]+::))
{ fail(::Run Name contains prohibited characters. Allowed characters are: a-z, A-Z, 0-9, -, _, and .::) }
```

  - Run Name must not exceed 255 characters.

```
if (step.::Run Name::.length() > 255) { fail(::Run Name shall not exceed 255 characters.::) };
```

- Checks the Index Reads, Index Read 1, and Index Read 2 field values.
  - If Index Reads is No Index, Index Read 1 and Index Read 2 values must be 0 (error results if it is otherwise).
  - If Index Reads is Single Index, Index Read 1 value must be greater than 0, and Index Read 2 values must be 0 (error results if it is otherwise).
  - If Index Reads is Dual Index, Index Read 1 and Index Read 2 values must be greater than 0 (error results if it is otherwise).

```
if (step.::Index Reads:: == ::No Index::)
{ if (step.::Index Read 1:: != 0 || step.::Index Read 2:: != 0) { fail(::Index Read 1 and Index Read 2 must be 0 if the Index Reads is No Index.::) } }
else
{ if (step.::Index Reads:: == ::Single Index::)
{ if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) { fail(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if the Index Reads is Single Index.::) } }
else
{ if (step.::Index Read 1:: == 0 || step.::Index Read 2:: == 0) { fail(::Index Read 1 and Index Read 2 must be greater than 0 if the Index Reads is Dual Index.::) } } }
```

- Checks the Paired End, Read 2 Cycles, and Index Read 2 field values.
  - If Paired End is set to True and Read 2 Cycles value is 0, an error is generated.

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  - If Paired End is set to False and Read 2 Cycles or Index Read 2 values are greater than 0, an error is generated.

```
if (step.::Paired End::.toBoolean())
{ if (step.::Read 2 Cycles:: == 0) { fail(::Read 2 Cycles must not be zero if it is Paired End read.::) }}
else
{if (step.::Read 2 Cycles:: != 0 || step.::Index Read 2:: != 0) {fail(::Read 2 Cycles and Index 2 Cycles must be 0 if it is not Paired End Read.:: )}}
```

- Checks the Adapter Sequence Read 1 and Adapter Sequence Read 2 field values.
  - Adapter Sequence Read 1 and Adapter Sequence Read 2 can only contain ACTG+ characters.

```
if (step.hasValue(::Adapter Sequence Read 1::) && !step.::Adapter Sequence Read 1::.matches(::[ACTG+]+::)) { fail(::Adapter Sequence Read 1 contains prohibited characters. Allowed characters are: ACTG+::) };
if (step.hasValue(::Adapter Sequence Read 2::) && !step.::Adapter Sequence Read 2::.matches(::[ACTG+]+::)) { fail(::Adapter Sequence Read 2 contains prohibited characters. Allowed characters are: ACTG+::) }
```

- Checks Override Cycles field value.
  - Override Cycles can only contain Y, N, I, U, 0–9, and semicolon characters.

```
if (step.hasValue(::Override Cycles::) && !step.::Override Cycles::.matches(::[YNIU0-9;]+::))
{ fail(::Override Cycles contains prohibited characters. Allowed characters are: Y, N, I, U, 0-9 and ;. Example: N1Y150;I8;I7N1;Y141U10.::) }
```

- When the Run Mode is Cloud, the script does the following actions:
  - Sets Local Analysis Workflow Versions to None.
  - If the Cloud Analysis Workflow Versions is None, the script displays the following error: `Invalid option selected for Cloud Analysis Workflow Versions field.`

```
if (step.::Run Mode:: == ::Cloud::) { step.::Local Analysis Workflow Versions:: = ::None::; if (step.::Cloud Analysis Workflow Versions:: == ::None::) { fail (::Invalid option selected for Cloud Analysis Workflow Versions field.::); }};
```

  - Due to automation script limitation, a validation rule resides in the backend service to perform the following action: If Cloud Analysis Workflow Versions is 3.7.4 or later, set FASTQ Compression Format to gzip.
- When the Run Mode is Local or Hybrid, the script does the following actions:
  - Sets Cloud Analysis Workflow Versions to None.
  - If Local Analysis Workflow Versions is None, the script displays the following error message: `Invalid option selected for Local Analysis Workflow Versions field.`

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```
if (step.::Run Mode:: == ::Local:: || step.::Run Mode:: == ::Hybrid::) {
step.::Cloud Analysis Workflow Versions:: = ::None::; if (step.::Local Analysis
Workflow Versions:: == ::None::) { fail(::Invalid option selected for Local
Analysis Workflow Versions field.::); }};
```

  - Due to automation script limitation, a validation rule resides in the backend service. If Local Analysis Workflow Versions is 3.7.4 or later, an error occurs if FASTQ Compression Format is None.

- Generates the sample sheet and/or creates the planned run on ICA.
  - Sample sheet is attached to the step.

```
/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/nextseq1k2k-
automation.jar
script:generate_v2_sample_sheet -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid2} -c {compoundOutputFileLuid0}
```

  - There is backend validation of the sample sheet content. This validation makes sure that the sample sheet is valid to set up a Local Mode run or is ready for DRAGEN applications. Refer to *NextSeq 1000/2000 Integration v2.4 Validation and Troubleshooting* on page 1512 for submitted sample name character restrictions.

Set Next Step Automation

Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol—AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3). The automation is automatically triggered on exit of the Record Details screen.

```
nextStep = ::ADVANCE::
```

Register Step Completed

Automatically triggered on exit of the step, this automation registers the completion time of the step by publishing messages to Clarity LIMS Product Analytics through the LINK service. This script is only used for Clarity LIMS Product Analytics support.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_
completed.groovy'
```

Master Step Fields

There are 24 fields defined on the Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3) step. These fields are required for sample sheet generation and planned run creation on ICA.

Table 317 Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3) Master Step Fields Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Run Name	Text	Required Field	
Instrument Type	Text Dropdown	Required Field	Presets • NextSeq1000 • NextSeq2000
Run Mode	Text Dropdown	Required Field	Presets • Local • Hybrid • Cloud
Paired End	Text Dropdown	Required Field	Presets • True • False
Read 1 Cycles	Numeric Dropdown	Required Field Custom Entries	Presets • 301 • 151 • 101 • 51
Read 2 Cycles	Numeric Dropdown	Required Field Custom Entries	Presets • 301 • 151 • 101 • 51
Index Reads	Text Dropdown	Required Field	Presets • No Index • Single Index • Dual Index
Index Read 1	Numeric Dropdown	Required Field Custom Entries	Presets • 0 • 6 • 8

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Index Read 2	Numeric Dropdown	Required Field Custom Entries	Presets • 0 • 6 • 8
Analysis Workflow	Text	Required Field Read Only	Default • GenerateFASTQ
Adapter Sequence Read 1	Text		
Adapter Sequence Read 2	Text		
Barcode Mismatches Index 1	Numeric Dropdown		Presets • 0 • 1 • 2
Barcode Mismatches Index 2	Numeric Dropdown		Presets • 0 • 1 • 2
Override Cycles	Text		
Cloud Run ID	Text	Read Only	Hidden - Used in creating planned run in ICA. Do not delete, rename, or modify. These actions will break the integration.

For Research Use Only. Not for use in diagnostic procedures.

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Use Custom Index Read 1 Primer	Toggle Switch		Default • None Set Hidden Custom primer option is currently not supported. Do not enable as it will break the integration.
Use Custom Index Read 2 Primer	Toggle Switch		Default • None Set Hidden Custom primer option is currently not supported. Do not enable as it will break the integration.
Use Custom Read 1 Primer	Toggle Switch		Default • None Set Hidden Custom primer option is currently not supported. Do not enable as it will break the integration.

For Research Use Only. Not for use in diagnostic procedures.

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Use Custom Read 2 Primer	Toggle Switch		Default • None Set Hidden Custom primer option is currently not supported. Do not enable as it will break the integration.
Instructions	Multiline Text	Read Only	Default • If GenerateFASTQ analysis is required, please select the DRAGEN version in Cloud Analysis Workflow Versions (for Cloud run mode) or Local Analysis Workflow Versions (for Local/Hybrid run mode) custom fields above.
Local Analysis Workflow Versions	Text Dropdown		Default • None
Cloud Analysis Workflow Versions	Text Dropdown		Default • None
FASTQ Compression Format	Text Dropdown	Required Field	Presets • gzip (default value) • DRAGEN • None

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Do not modify the configuration of the Local Analysis Workflow Versions and Cloud Analysis Workflow Versions fields. Modifying these fields can break the integration.

User Interaction

Refer to *NextSeq 1000/2000 Integration v2.4 Validation and Troubleshooting* on page 1512 for details.

Step 3: AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3)

This step is fully automated.

The integration starts and completes the step automatically. Data from the run is parsed back to Clarity LIMS. No user interaction is required. In this step, the pooled samples in the reagent cartridge are sequenced on the NextSeq 1000/2000 instrument.

Do not add samples to the Ice Bucket or start or complete the step. The integration completes these actions automatically.

Register Step Started & Register NextSeq1k2k Run & Register NextSeq1k2k Run Association & Register NextSeq1k2k RunStarted

Automatically triggered at the beginning of the step, this automation completes the following actions:

- Registers the start time of the step.
- Registers the NextSeq 1000/2000 sequencing run configuration.
- Registers the association of the sequencing run with the actual samples by linking the Instrument Run ID with the IDs for the samples.
- Registers the time that the sequencing run starts.

The automation completes these actions by publishing messages to Clarity LIMS Product Analytics through the LINK service. This automation is only used for Clarity LIMS Product Analytics support.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/common/step_
started.groovy' && /opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/unified-product-analytics/automation/unified-
product-analytics-automation.jar script:executeUPAAutomationScript -i
{stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0} -s
'com/illumina/upa/scripts/nextseq1k2k/register_nextseq1k2k_run.groovy' &&
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/nextseq1k2k/nextseq1k2k_
```

```
associate_seqrun.groovy' && /opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/unified-product-analytics/automation/unified-
product-analytics-automation.jar script:executeUPAAutomationScript -i
{stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0} -s
'com/illumina/upa/scripts/nextseq1k2k/nextseq1k2k_seqrun_started.groovy'
```

Register NextSeq1k2k RunCompleted & Register Step Completed

Automatically triggered on exit of the step, this automation completes the following actions:

- Registers the sequencing run completion time, run status, and run metrics. This script requires the Cloud Run ID field from the Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3) step.
- Registers the completion time of the step.

The automation completes these actions by publishing messages to Clarity LIMS Product Analytics through the LINK service. This automation is only used for Clarity LIMS Product Analytics support.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/nextseq1k2k/nextseq1k2k_
seqrun_completed.groovy' && /opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/unified-product-analytics/automation/unified-
product-analytics-automation.jar script:executeUPAAutomationScript -i
{stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0} -s
'com/illumina/upa/scripts/common/step_completed.groovy'
```

Master Step Fields

There following fields are defined on the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3) step. These fields are used to display the run status and sequencing run and analysis configuration parsed from the `RunParameters.xml` file of the sequencing run.

Do not modify the master step field names. This action causes the integration to break.

Run Parameters and Corresponding Clarity LIMS Step Fields

The following table shows how some of the step fields map to the fields on the `RunParameters.xml` file, and whether the field is visible on the Record Details screen.

Master Step Field	RunParameters.xml Field	On Record Details Screen
Current Cycle	Calculated based on CompletedCycles Field	Visible
Current Read	Calculated based on CompletedCycles field against PlannedCycles	Visible
Flow Cell ID	FlowCellSerialNumber	Visible
Flow Cell Lot Number	FlowCellLotNumber	Visible
Instrument Control Software Version	ApplicationVersion	Visible
Instrument ID	InstrumentSerialNumber	Visible
Output Folder	OutputFolder	Visible
Reagent Cartridge ID	CartridgeSerialNumber	Visible
Reagent Cartridge Lot Number	CartridgeLotNumber	Visible
RTA Version	RtaVersion	Visible
Run Name	ExperimentName	Visible
Secondary Analysis Workflow	SecondaryAnalysisWorkflow	Visible
Flow Cell Mode	FlowCellMode	Hidden Do not delete, rename, or modify. These actions can break the integration.
Instrument Run ID	Derived from OutputFolder	Hidden Do not delete, rename, or modify. These actions can break the integration.

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Master Step Field	RunParameters.xml Field	On Record Details Screen
Run End Time	RunEndTime	Hidden Do not delete, rename, or modify. These actions can break the integration.
Run Start Time	RunStartTime	Hidden Do not delete, rename, or modify. These actions can break the integration.
Secondary Analysis Mode	SecondaryAnalysisMode	Hidden Do not delete, rename, or modify. These actions can break the integration.
Secondary Analysis Platform Version	SecondaryAnalysisPlatformVersion	Hidden Do not delete, rename, or modify. These actions can break the integration.
SkipObdd	SkipObdd	Hidden Do not delete, rename, or modify. These actions can break the integration.

Additional Master Step Fields and Values

The following table shows how the other step fields derive their values, and whether the step field is visible on the Record Details screen.

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Master Step Field	RunParameters.xml Field	On Record Details Screen
Instrument Platform	NextSeq 1000/2000 Constant value	Visible
Instrument Type	One of the following options: • NextSeq1000 • NextSeq2000 Determined from Instrument Type step field from previous Load to Reagent Cartridge step.	Visible
Run Status	One of the following options: • RunStarted • RunCompletedSuccessfully • RunAbortedByUser • RunErroredOut Set by the SIS integration service[1]	Visible
Sequencing Log	Filled in by the SIS integration service[1] as the sequencing run proceeds	Visible
BaseSpace Run ID	Received from SQS message in the SIS integration service[1]	Hidden Do not delete, rename, or modify. These actions will break the integration.

[1]For information on how the integration works, refer to *Run Status, Primary Metrics, and Analysis Results Parsing and Recording* in *How the NextSeq 1000/2000 Integration Works* on page 1502.

Global Fields

The following global fields are used to capture the run metrics in Clarity LIMS.

- % Bases >=Q30 R1
- % Bases >=Q30 R2
- % Error Rate R1
- % Error Rate R2
- Yield (Gb) R1
- Yield (Gb) R2

- Reads PF R1
- Reads PF R2
- %PF R1
- %PF R2
- % Aligned R1
- % Aligned R2
- % Phasing R1
- % Phasing R2
- % PrePhasing R1
- % PrePhasing R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- Cluster Density R1
- Cluster Density R2

At the end of this step, the pool of samples is automatically advanced to (and queued for) the Demultiplexing (NextSeq 1000/2000 Sequencing v2.3) step.

Step 4: Demultiplexing (NextSeq 1000/2000 Sequencing v2.3)

This step is a semi-automated step.

The integration starts the step automatically and demultiplexing data from the GenerateFASTQ secondary analysis is parsed back to Clarity LIMS. The lab scientist reviews the demultiplexing result parsed into Clarity LIMS, assigns QC flags, and completes the step.

For the demultiplexing information in the Sample Details table to display correctly, the following conditions must be met:

- The DRAGEN version must be earlier than v3.9.3.
- The samples in the run must not repeat.
- The run must be for the single lane flow cell (P1 or P2).

Do not add samples to the Ice Bucket or start or complete the step. The integration completes these actions automatically.

Register Step Started Automation

Automatically triggered at the beginning of the step, this automation registers the start time of the step by publishing messages to Clarity LIMS Product Analytics through the LINK service. This automation is only used for Clarity LIMS Product Analytics support.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_started.groovy'
```

Assign Demultiplexing QC Flags Automation

Automatically triggered when you select a button on the Record Details screen, this automation assigns QC flags based on the criteria set in the step fields.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -u {username} -p {password} script:assignQCForDemuxStep -i {processURI:v2} -log {compoundOutputFileLuid1} -qcResult {compoundOutputFileLuid2}
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation sets the next step to Advance and the samples to complete the protocol.

```
nextStep = ::ADVANCE::
```

Register Analysis Completed & Register Step Completed Automation

Automatically triggered on exit of the step, this automation performs the following actions:

- Registers the NextSeq 1000/2000 analysis status and result.
- Registers the completion time of the step.

These actions are done by publishing messages to Clarity LIMS Product Analytics through the LINK service. This automation is only used for Clarity LIMS Product Analytics support.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_completed.groovy' && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/nextseq1k2k/nextseq1k2k_analysisrun_completed.groovy'
```

Master Step Fields

The following table lists the 12 master step fields that are configured on the Demultiplexing (NextSeq 1000/2000 Sequencing v2.3) master step.

Table 318 Master Step Field Configuration for Demultiplexing (NextSeq 1000/2000 Sequencing v2.3) Step

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Criteria 1 - Source Data Field	Text Dropdown	Custom Entries	Presets • # Reads • # Perfect Index Reads • # One Mismatch Index Reads • # of >= Q30 Bases (PF)
Criteria 2 - Source Data Field	Text Dropdown	Custom Entries	Presets • # Reads • # Perfect Index Reads • # One Mismatch Index Reads • # of >= Q30 Bases (PF)
Criteria 1 - Operator	Text Dropdown	Custom Entries	Presets • >= • <= • = • !=
Criteria 2 - Operator	Text Dropdown	Custom Entries	Presets • >= • <= • = • !=
Criteria 1 - Threshold Value	Numeric		Valid integer value

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Criteria 2 - Threshold Value	Numeric		Valid integer value
Log	Multiline Text	Read Only	
Analysis Completed Time	Text	Read Only	Hidden Do not delete, rename, or modify. These actions will break the integration.
Analysis Started Time	Text	Read Only	Hidden Do not delete, rename, or modify. These actions will break the integration.
Analysis Status	Text	Read Only	Hidden Do not delete, rename, or modify. These actions will break the integration.
BSSH Analysis ID	Text	Read Only	Hidden Do not delete, rename, or modify. These actions will break the integration.

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Reagent Cartridge ID	Text	Read Only	Hidden Used for parsing demultiplexing results Do not delete, rename, or modify. These actions will break the integration.

Global Fields

The following table lists the global fields that are configured on the Demultiplexing (NextSeq 1000/2000 Sequencing v2.3) step. These fields are used to display the demultiplexing result metrics for individual library in the sample pool.

Table 319 Global Field Configuration for Demultiplexing (NextSeq 1000/2000 Sequencing v2.3) Step

Field Name	Field Type	Field Constraints/Options
# of >= Q30 Bases (PF)	Numeric	Read Only
# One Mismatch Index Reads	Numeric	Read Only
# Perfect Index Reads	Numeric	Read Only
# Reads	Numeric	Read Only
Mean Quality Score (PF)	Numeric	Read Only

User Interaction

Refer to *NextSeq 1000/2000 Integration v2.4 Validation and Troubleshooting* on page 1512 for details.

How the NextSeq 1000/2000 Integration Works

Sample Sheet Generation and Planned Run Creation

On the Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3) step, when the Validate Run Setup and Create Planned Run automation is triggered, the run and analysis parameters entered in the Run Details screen are sent to Illumina Connected Analytics (ICA). ICA validates the run and analysis configuration, and sends an error message to Clarity LIMS if the validation fails. If the validation passes, ICA generates the sample sheet (and sends back to Clarity LIMS) and/or creates a planned run depending on the Run Mode selected.

- Local run mode: Only the sample sheet is generated and stored on Clarity LIMS. The sample sheet contains all the run and analysis configuration required to start the run on the instrument.
- Hybrid/Cloud run mode: A planned run is created on ICA. This planned run contains all the run and analysis configuration required to start the run on the instrument. The sample sheet is also generated and stored on Clarity LIMS for reference purposes.

Refer to *Start A Sequencing Run On Instrument With Different Run Modes* on page 1556 for details on how to start a run using different run mode.

Run Status, Primary Metrics, and Analysis Results Parsing and Recording

After the sequencing run is started on the instrument, NextSeq 1000/2000 Control Software (NCS) notifies BaseSpace Sequence Hub that the sequencing run has started.

BSSH then does the following actions:

- Local run mode: Creates a run on the BaseSpace Sequence Hub database with the run configuration information received from NCS.
- Hybrid/Cloud run mode: Creates a run on the BaseSpace Sequence Hub database by copying the run configuration information from the planned run created in ICA, using a special Cloud Run ID received from NCS.

After the run is created on BaseSpace Sequence Hub, a run update event message that carries the RunStarted equivalent message is created on BaseSpace Sequence Hub and delivered through ICA and, eventually, to Clarity LIMS SQS queue service. The SIS integration service captures and processes the message.

Based on the reagent cartridge barcode information in the event message (refer to the following example), SIS looks in the queue of AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3) step for a container with the same reagent cartridge barcode. When identified, the step is started automatically and the Run Status field on Record Details screen is updated to RunStarted. SIS then updates all the other step fields (eg, Current Read, Output Folder) by parsing the details from the `RunParameters.xml` uploaded to BaseSpace Sequence Hub by NCS.

```
#Example of an run update event
{
"acl": [
```


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```
"wid:61dc14e8-d823-3bd1-bea3-66f9c8388254"
],
"apiUrl": "https://api.basespace.illumina.com/v2/runs/r.ZvTfDXsNhUyh2kA5S4Zewg",
"dateModified": "2020-03-26T09:19:58.5546497Z",
"flowcellBarcode": "11M11COMC",
"gdsFolderPath": "/Runs/200326_MP2-15_87_11M11COMC_r.ZvTfDXsNhUyh2kA5S4Zewg",
"gdsVolumeName": "bssh.61dc14e8d8233bd1bea366f9c8388254",
"id": "r.ZvTfDXsNhUyh2kA5S4Zewg",
"instrumentRunId": "200326_MP2-15_87_11M11COMC",
"name": "200326_MP2-15_87_11M11COMC",
"reagentBarcode": "EC0001990-EC5",
"sampleSheetName": "SampleSheet.csv",
"status": "Running",
"v1pre3Id": "194424236"
}
```


How the SIS Integration Service Handles Run Status

The NCS continues to notify BaseSpace Sequence Hub of any run status change throughout the course of the sequencing run. The run update event delivery process continues as described in the previous section.

The following table lists the run status details and how the SIS integration service handles each status.

Run Status	Shown in Step	Details
RunStarted	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3)	Run Status field is updated to RunStarted. All other step fields (except Run End Time) are updated based on `RunParameters.xml` detail obtained from BaseSpace Sequence Hub, the SQS event message, and the step field value from the Load to Reagent Cartridge (NextSeq 1000/2000 v2.3) step. Reagent cartridge lot information received from BaseSpace Sequence Hub is compared against the details of the pre-accessioned reagent cartridge on Clarity LIMS. • If the expiration date is different, it is updated to the date received from BaseSpace Sequence Hub. • If reagent cartridge is not found, it is created and set to Active. • If reagent cartridge is not in Active status, it is set to Active. Multiline Sequencing Log field is updated.

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Run Status	Shown in Step	Details
RunCompletedSuccessfully	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3)	Run Status field is updated to RunCompletedSuccessfully Current Read and Current Cycle fields are updated to the final cycle number and read number based on `RunParameters.xml` detail obtained from BaseSpace Sequence Hub. Run End Time field is updated based on RunParameters.xml detail obtained from BaseSpace Sequence Hub. Sequencing run result (primary metrics) is downloaded from BaseSpace Sequence Hub and the fields in Sample Details table (eg, % Error Rate R1, % Phasing, etc.) are updated with the values. Multiline Sequencing Log field is updated. SIS autocompletes the step and routes the samples in the reagent cartridge container to the next step (eg, Demultiplexing (NextSeq 1000/2000 Sequencing v2.3)) step.

Run Status	Shown in Step	Details
RunErroredOut	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3)	Run Status field is updated to RunErroredOut. Current Read and Current Cycle fields are updated to the final cycle number and read number that the run stopped based on `RunParameters.xml` detail obtained from BaseSpace Sequence Hub. Run End Time field is updated based on `RunParameters.xml` detail obtained from BaseSpace Sequence Hub. Sequencing run result (primary metrics) is downloaded from BaseSpace Sequence Hub and the fields in Sample Details table (eg, % Error Rate R1, % Phasing, etc.) are updated with the values. Multiline Sequencing Log field is updated. SIS does not autocomplete the step. Complete the step manually.

Run Status	Shown in Step	Details
RunAbortedByUser	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3)	Run Status field is updated to RunAbortedByUser. Current Read and Current Cycle fields are updated to the final cycle number and read number that was aborted based on `RunParameters.xml` details obtained from BaseSpace Sequence Hub. Run End Time field is updated based on RunParameters.xml detail obtained from BaseSpace Sequence Hub. Sequencing run result (primary metrics) is downloaded from BaseSpace Sequence Hub and the fields in Sample Details table (eg, % Error Rate R1, % Phasing, etc.) are updated with the values. Multiline Sequencing Log field is updated. SIS does not autocomplete the step. Complete the step manually.
AnalysisStarted	Demultiplexing (NextSeq 1000/2000 Sequencing v2.3)	SIS starts the step automatically for the reagent cartridge container. Multiline Sequencing Log field is updated.

Run Status	Shown in Step	Details
AnalysisCompleted	Demultiplexing (NextSeq 1000/2000 Sequencing v2.3)	SIS downloads the `Demultiplex_stats.csv` file, which contains the demultiplexing result from BaseSpace Sequence Hub. The file is parsed and details are recorded in the Sample Details table for each library in the library pool. Hidden master step fields are updated with information from BaseSpace Sequence Hub. In particular, Analysis Status is updated to Complete. Multiline Sequencing Log field is updated. SIS does not autocomplete the step. Assign the QC flag to the individual library and manually complete the step.
AnalysisFailed	Demultiplexing (NextSeq 1000/2000 Sequencing v2.3)	Hidden step fields are updated with information from BaseSpace Sequence Hub. In particular, Analysis Status is updated to either TimedOut or Aborted. Multiline Sequencing Log field is updated. SIS does not auto-complete the step. Assign the QC flag to the individual library and manually complete the step.

Start A Sequencing Run On Instrument With Different Run Modes

Local Run Mode

1. On the NextSeq 1000/2000 Control Software (NCS) interface, select the menu button and select Settings.
2. Select Local Run Setup and Proactive, Run Monitoring and Storage*.
3. [Optional] Enter the default output folder location.
4. Select Save.
5. On the main screen, select Start.
6. Sign in to the BaseSpace Sequence Hub (BSSH) account that was used during NextSeq 1000/2000 Integration package installation setup.

a. Select the workgroup (if applicable).
b. Select Next.

7. Select Choose, and a file browser pops up.
8. Browse and select the sample sheet generated during the Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3) step.
   When selected, the sample sheet has its content validated. After the validation passes, run and analysis configuration is parsed and autopopulated in the next page.
9. Select Review to review the sequencing run settings.
10. Select Load and manually load the reagent cartridge and flow cell.
11. Select Sequence and the pre-run check proceeds.
    After the pre-run check passes, the sequencing run starts automatically.

Hybrid or Cloud Run Mode

1. On the NextSeq 1000/2000 Control Software interface, select the menu button and select Settings.
2. Select Online Run Setup and Proactive, Run Monitoring and Storage*.
3. [Optional] Enter the default output folder location.
4. Select Save.
5. On the main screen, select Start.
6. Sign into the BSSH account that was used during NextSeq 1000/2000 Integration package installation setup.
   a. Select the workgroup (if applicable).
   b. Select Next.
      Planned runs that were created in the Load to Reagent Cartridge (NextSeq 1000/2000 v2.3) step using the Hybrid or Cloud run modes are displayed.
7. Select the run and select Review to review the sequencing run settings.
8. Select Load and manually load the reagent cartridge and flow cell.
9. Select Sequence and the pre-run check proceeds.
   After the pre-run check passes, the sequencing run starts automatically.

Components Installed

The following sections describe the various components that are installed by default as part of this integration. These components include the following items:

- Files
- Properties
- Reagent categories/label groups
- Reagent kits

- Control types
- Containers

Information on installed workflows, protocols, steps, and automation points is provided in the Workflows, Protocols, and Steps section of *NextSeq 1000/2000 Integration v2.4 Validation and Troubleshooting* on page 1512.

Reagent Categories/Label Groups

- TruSeq HT adapters v2 (D7-D5)

Reagent Kits

- Resuspension Buffer (RSB)
- NextSeq 1000/2000 reagent cartridge

Container Types

- Tube
- 96-well plate
- NextSeq 1000/2000 reagent cartridge

Control Types

- PhiX v3

This integration supports the NextSeq 1000/2000 reagent cartridge with barcode provided in the format [A-Za-z]{2}[0-9]{7}-[A-Za-z0-9]{4} (eg, EC1234567-EC03).

Re-queue Samples for Cloud Run

Clarity LIMS requires unique library names when samples are re-queued to the workflow in the NextSeq 1000/2000 integration. Assign unique names to libraries after going through library preparation.

The following steps are used to configure the library preparation workflow correctly before routing the samples for re-queuing:

1. From Configuration, select the Lab Work tab.
2. Search for the library preparation workflow used for the re-queued samples.
3. In the Master Step of the library preparation workflow, modify the naming convention under Step Type to generate unique library names (eg, appending LIMS ID to the default naming convention, like OutputItemLIMSID).
4. Select Save.

Rules and Constraints

The NextSeq 1000/2000 Reagent Cartridge barcode should not be modified after a successful validation. Modifications can cause issues when Clarity LIMS tries to update the status and sample details of subsequent steps.

- The workflow configuration contains several validation checks. To make sure that the calculations work properly, it is important that you do not disable any of this validation logic. The validation checks determine:
  - Which samples, and how many, can enter each step together.
  - Which samples, and how many, can be pooled together.
- The NextSeq 1000/2000 reagent cartridge barcode must be unique. There should not be multiple NextSeq 1000/2000 reagent cartridge containers in the system with the same name.
- Reagent labels (indexes) must be unique.
- One library pool can only contain one library or control with no label (index).
- Do not manually start or complete the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3) step. This step is a fully automated step, and the SIS integration service does not update samples correctly if they have been manually started.
- Do not manually start the Demultiplexing (NextSeq 1000/2000 Sequencing v2.3) step. This step is semi-automated, and the SIS integration service does not update the demultiplexing result correctly if they have been manually started.
- For the automated run to start successfully, select Validate Run Setup and Create Planned Run in the Load to Reagent Cartridge step.

NextSeq 1000/2000 Integration v2.4 Validation and Troubleshooting

This section explains how to validate the installation of the Illumina NextSeq 1000/2000 Integration Package v2.4.

The validation process involves the following items:

- Running samples through the Library Prep Validation v2.3.1 workflow.
  - The workflow contains a single-step protocol that models the library prep workflow required to produce libraries tagged with index sequences. At the end of the step, these libraries are routed to NextSeq 1000/2000 Sequencing v2.3 workflow.
- Running the libraries through the NextSeq 1000/2000 Sequencing v2.3 workflow. This process validates the following information:
  - Successful sequential step advancement of samples in the following steps:
    1. Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3)
    2. Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3)
    3. AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3)
    4. Demultiplexing (NextSeq 1000/2000 Sequencing v2.3)

- Automatic validation of run setup information before sample sheet generation or planned run creation.
- Automated generation of a sample sheet. The sample sheet is used to start the sequencing run on NextSeq 1000/2000 Control Software (NCS) via Local run mode.
- Automated creation of a planned run on Illumina Connected Analytics. The NextSeq 1000/2000 Control Software retrieves the planned run to start the run via Hybrid or Cloud run mode.
- Automated tracking of the NextSeq 1000/2000 sequencing run and parsing of run statistics from BaseSpace Sequence Hub into Clarity LIMS using the SIS Integration Service.
- Automated tracking of the secondary analysis using DRAGEN (up to BCL Convert only).
- Parsing of demultiplexing metrics from BaseSpace Sequence Hub into Clarity LIMS using SIS integration service.

Before running the validation steps below, these steps assume that the NextSeq 1000/2000 Integration Package v2.4 is installed, and that you have imported the default Clarity LIMS configuration.

For information on how the integration works, refer to *NextSeq 1000/2000 Integration v2.4 Configuration and User Interaction* on page 1475.

Activate Workflow, Create Project, Add and Assign Samples

The following steps set up Clarity LIMS in preparation for running samples through the Library Prep Validation v2.3.1 and NextSeq 1000/2000 Sequencing v2.3 workflows.

1. On the Configuration tab, under Workflows, activate both the Library Prep Validation v2.3.1 and NextSeq 1000/2000 Sequencing v2.3 workflows.
2. On the Projects and Samples screen, create a project and add samples to it.

   Use only alphanumeric, dash, and underscore characters in the submitted sample names. Failing to do so causes sample sheet validation failure in Load To Reagent Cartridge step.

3. Assign the samples to the Library Prep Validation v2.3.1 workflow.

Library Prep Protocol: Library Prep Validation v2.3.1

This single-step protocol models the library prep required to produce libraries tagged with index sequences that are ready for the NextSeq 1000/2000 Sequencing v2.3 workflow.

Run Library Prep Validation v2.3.1 Step

1. In Lab View, locate the Library Prep Validation v2.3.1 protocol.
   Samples are queued for the Library Prep Validation v2.3.1 step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select the label groups to use.
   - TruSeq HT Adapters v2 (D7-D5) is the default label group delivered by Library Prep Validation v2.3.1 protocol.

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- For other reagent labels (indexes), manually create them in the Configuration screen under Consumables > Labels.

  For more information on how to create reagent label group, refer to the *Add and Configure Labels and Label Groups* section in the *Clarity LIMS Core Functionality* documentation.

4. Select Begin Work.
5. On the Placement screen, perform the following actions:
   a. In the Placed Samples area in the Container field, scan or enter the barcode of the destination container.
   b. Select the samples from the Samples to be Placed area, then drag them over to the container wells on the right.
   c. Select Add Labels.
6. On the Add Labels screen, the Labels to Add list is populated with labels from the TruSeq HT Adapters v2 (D7-D5) label group. This label group is the only one configured in the out-of-the-box integration. It is used by default in this step.
   a. Select labels on the left, and drag them over to the container on the right to assign them to placed samples.
   b. Select Record Details.
7. On the Record Details screen, perform the following actions:
   a. In the Sample Details table, enter values for Molarity (nM) for all the individual sample. Enter values individually or use the copy/paste functionality to enter multiple values from a spreadsheet.
      - Molarity (nM) values are used in Calculate Volume automation script in Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3) step.

        If Molarity (nM) values are not entered, the Calculate Volume automation script does not work properly.

   b. The Sequencing Instrument column indicates the instrument to be used for the run. Select NextSeq 1000/2000.
   c. Populate the fields in this column using one of the following methods:
      - To populate fields individually for each sample, select from the drop-down list in each row.
      - To populate fields all at one time, use the Copy and Paste buttons to enter multiple values from a spreadsheet (refer to the following note).

      Alternatively, select inside the table and press Ctrl + A to select all rows. At the top of the table, select Sequencing Instrument, then select NextSeq 1000/2000 from the adjacent drop-down list. Select Apply.

      If working with many samples, copy and paste to insert multiple values from a spreadsheet as follows.

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a. Select the Copy button to copy the sample information from Clarity LIMS to the clipboard.
b. Open a blank Excel document and paste the contents of the clipboard into the spreadsheet.
c. In the spreadsheet, enter the Molarity (nM) and Sequencing Instrument values. Select the entire spreadsheet, including the headers, (Ctrl + A) and copy (Ctrl + C) the information to the clipboard.
d. In Clarity LIMS, select the Paste button. Paste the contents of the clipboard (Ctrl + V) into the Update Table window and then select Update to paste into the Sample Details table.

8. Select Next Steps.
   This selection triggers the Next Steps automation, which sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step successfully.
9. On the Assign Next Steps screen in the Samples table, the Next Step for all samples is prepopulated with Remove from workflow.

   Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.

10. Select Finish Step.
    On exit from the step, the Routing Script automation is triggered. This automation assigns samples to the first step of the NextSeq 1000/2000 Sequencing v2.3 workflow, which is Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3) step.

NextSeq 1000/2000 Sequencing v2.3 Protocol

Step 1: Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3)

1. In Lab View, locate the NextSeq 1000/2000 Sequencing v2.3 protocol. The samples are queued for the Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Pooling screen, perform the following actions:
   a. Create a pool by dragging samples into the Pool Creator.

      Do not create more than one pool for this step. The Calculate Volumes automation in this step supports one pool only.

   b. Type a name for the pool or accept the default name (Pool #1).
   c. Select Record Details.

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On the Record Details screen, the Reagent Lot Tracking section tracks the Resuspension Buffer (RSB) lot information used in the step.

5. To add and activate additional reagent lots, perform the following steps:
   a. In a new browser tab, open an additional instance of Clarity LIMS.
   b. Navigate to the Consumables configuration screen.
   c. Select the RSB reagent kit and select New Lot.
   d. For Status of Reagent Lot, select Active.
   e. Enter the lot details.
   f. Select Save.
6. In the Reagent Lot Tracking section, select from the active lots displayed in the drop-down list.
   The Step Details area contains two required fields:
   - Final Loading Concentration (pM)—The value entered in this field is the recommended final loading concentration specified in the *Illumina NextSeq 1000/2000 Sequencing System Guide* at `support.illumina.com`. The value depends on the library type. The default drop-down list contains the values 650, 750, 1000, and 2000. A custom value is acceptable.
   - Final Loading Volume (µl)—The value in this field is the final loading volume of the pool into the reagent cartridge. The field is prepopulated with the configured default value, 24 µl, specified in the *Illumina NextSeq 1000/2000 Sequencing System Guide* at `support.illumina.com`. The value is editable when more volume is necessary.
7. Select Calculate Volumes.
   This selection triggers the Calculate Volumes automation. This automation calculates the volume required for each library to form a pool that has the concentration and volume specified in the step details fields.
   The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains volume information of each of the samples and RSB buffer to add to the pool. Select the file to download it, then open it in Excel.
8. In the Sample Details table, select the pool icon to view details on the pool composition.
9. Select Next Steps.
   This selection triggers the Set Next Step automation, which sets the next step for samples to ADVANCE, advancing them to the next step in the protocol. The next step is Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3).
   On the Assign Next Steps screen, the next step for samples is already set to the next step in the workflow. The next step is Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3).
10. Select Finish Step.

At the end of this step, the pool of samples automatically advances to Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3) step.

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Step 2: Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3)

1. In Lab View, locate the NextSeq 1000/2000 Sequencing v2.3 protocol. The pool of samples is queued for the Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
   The Validate Single Input automation is triggered. This automation checks that there is only one container input to the step.
4. On the Placement screen, perform the following actions:
   a. Drag the pool into the NextSeq 1000/2000 Reagent Cartridge field in the Placed Samples area.
   b. Scan or type the barcode of the reagent cartridge into the NextSeq 1000/2000 Reagent Cartridge field.
   c. Select Record Details.
   On exit of the Placement screen, the Validate Reagent Cartridge Barcode automation checks that the reagent cartridge barcode conforms to the barcode mask [A-Za-z]{2}[0–9]{7}-[A-Za-z0-9]{4}. If not, an error message displays.

   > The NextSeq 1000/2000 Reagent Cartridge barcode should not be modified after a successful validation. Modifications can cause issues when Clarity LIMS tries to update the status and sample details of subsequent steps.

   On entry to the Record Details screen, the Retrieve Analysis Workflow Versions automation fetches the available analysis workflow versions from ICA. It then updates the preset values of both Local Analysis Workflow Versions & Cloud Analysis Workflow Versions field.
5. On the Record Details screen, the Reagent Lot Tracking section tracks the NextSeq 1000/2000 Reagent Cartridge lot information used in the step. Follow the steps in Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3) step if you must add a lot.
6. In the Reagent Lot Tracking section, select from the active lots displayed in the drop-down list.
7. The fields displayed on the Record Details screen are used to create planned run and generate the sample sheet file.
   - Run Name—Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.
   - Instrument Type—Select from preset options (NextSeq 1000 or NextSeq 2000).
   - Run Mode—Select from preset options: Local, Hybrid, Cloud. For more information on the different run modes, refer to *Run Mode Details* on page 1519.
   - Paired End—Select from preset options (True or False).
   - Read 1 Cycles—Select from preset options (301, 151, 101, 51) or type a custom value.
   - Read 2 Cycles—Select from preset options (301, 151, 101, 51) or type a custom value.

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- Index Reads—Select from preset options (No Index, Single Index, Dual Index).
- Index Read 1—Select from preset options (0, 6, 8) or type a custom value.
- Index Read 2—Select from preset options (0, 6, 8) or type a custom value.
- Analysis Workflow—Read only. This field is set to GenerateFASTQ.
- Local Analysis Workflow Versions—[Optional] (default is None). Only mandatory when Run Mode = Local/Hybrid and Analysis Workflow = GenerateFASTQ. Select according to the DRAGEN version installed on the instrument.
- Cloud Analysis Workflow Versions—[Optional] (default is None). Only mandatory when Run Mode = Cloud and Analysis Workflow = GenerateFASTQ. Select the cloud DRAGEN version to be used.
- FASTQ Compression Format:
  - If Run Mode = Local/Hybrid, Analysis Workflow = GenerateFASTQ, and Local Analysis Workflow Version >= 3.7.4, select gzip or DRAGEN.
  - If Run Mode = Cloud, Analysis Workflow = GenerateFASTQ, and Local Analysis Workflow Version >= 3.7.4, field is set to gzip automatically.
  - Otherwise, select None.
- Adapter Sequence Read 1—[Optional] Enter the Read 1 adapter sequence of the index adapter kit.
- Adapter Sequence Read 2—[Optional] Enter the Read 2 adapter sequence of the index adapter kit.
- Barcode Mismatches Index 1—[Optional] Select from preset options (0, 1, 2). Leave it blank if you are unsure.
- Barcode Mismatches Index 2—[Optional] Select from preset options (0, 1, 2). Leave it blank if you are unsure.
- Override Cycles—[Optional] String used to specify UMI cycles and mask out cycles of a read (eg, N1Y150;I8;I7N1;Y141U10). Leave it blank if you are unsure.
- Instructions — Read only.

8. On the Record Details screen, select Validate Run Setup and Create Planned Run.
   This selection triggers the automation script, which does the following actions:
   - Validates the parameters entered on the Record Details screen.
   - Create the planned run and send it to ICA if Run Mode is Hybrid or Cloud.
   - Generates the sample sheet and attaches it to the placeholder in the Files area of the Record Details screen for all Run Mode types.
9. Select Next Steps.

On the Assign Next Steps screen, the next step for samples is set to the next step in the workflow. The next step is AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3).

10. Select Finish Step.

At the end of this step, the pool of samples automatically advances to and is queued for the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3) step. The sequencing run is ready to be started. For details on how to start the sequencing run for different run modes, refer to *NextSeq 1000/2000 Integration v2.4 Configuration and User Interaction* on page 1475.

Run Mode Details

The run mode applies to all runs and determines how the run and analysis parameters are configured and how to analyze data.

- Local Mode—NextSeq 1000/2000 Integration service generates the sample sheet. Run/Analysis configuration is imported manually into NextSeq 1000/2000 instrument via a sample sheet to start the run. The downstream secondary analysis is done using the onboard DRAGEN module.
- Hybrid Mode—NextSeq 1000/2000 Integration service sends the run/analysis configuration to Illumina Connected Analytics. Run/Analysis configuration is downloaded to NextSeq 1000/2000 instrument via ICA to start the run. The downstream secondary analysis is done using the onboard DRAGEN module.
- Cloud Mode—NextSeq 1000/2000 Integration service sends the run/analysis configuration to ICA. Run/Analysis configuration is downloaded to NextSeq 1000/2000 instrument via ICA to start the run. The downstream secondary analysis is performed in the cloud.

Step 3: AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3)

This step is fully automated.

Do not add samples to the Ice Bucket or start or complete the step. The integration completes these actions automatically.

The integration starts the step automatically and data from the run is parsed back to Clarity LIMS. No user interaction is required. However, you can open and review the various stages of the step in Clarity LIMS. Do not perform any action when reviewing the data.

Read summary metrics are recorded for the library pool in the Step Details section and the Sample Details table.

Values are populated in the following master step fields:

- Run Name
- Current Read
- Flow Cell ID
- Reagent Cartridge Lot Number

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- Instrument ID
- Run Status
- Current Cycle
- Flow Cell Lot Number
- Instrument Platform
- Instrument Control Software Version
- Output Folder
- Secondary Analysis Workflow
- Reagent Cartridge ID
- Instrument Type
- RTA Version
- Sequencing Log

The summary metrics (per run level) populate in the following global fields.

- % Bases >=Q30 R1
- % Bases >=Q30 R2
- % Error Rate R1
- % Error Rate R2
- Yield (Gb) R1
- Yield (Gb) R2
- Reads PF R1
- Reads PF R2
- %PF R1
- %PF R2
- % Aligned R1
- % Aligned R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- Cluster Density R1

- Cluster Density R2

At the end of this step, the pool of samples automatically advances to (and queues for) the Demultiplexing (NextSeq 1000/2000 Sequencing v2.3) step.

Step 4: Demultiplexing (NextSeq 1000/2000 Sequencing v2.3)

This step is semi-automated.

For the demultiplexing information in the Sample Details table to display correctly, the following conditions must be met:

- The DRAGEN version must be earlier than v3.9.3.
- The samples in the run must not repeat.
- The run must be for the single lane flow cell (P1 or P2).

Do not add samples to the Ice Bucket or start or complete the step. The integration completes these actions automatically.

The integration starts the step automatically and demultiplexing data from the GenerateFASTQ secondary analysis is parsed back to Clarity LIMS. Review the data parsed and assign QC, depending on the criteria set, and complete the step.

In the Record Details screen, perform the following actions:

1. Review demultiplexing data.
   - Demultiplexing metrics are recorded for the library pool in the Sample Details table. Metrics include columns for # Reads, # Perfect Index Reads, # One Mismatch Index Reads, and # of >= Q30 Bases (PF).
   - The `Demultiplex_stats.csv` file generated by the DRAGEN onboard or Cloud analysis module is stored under the Files section.
2. Assign QC flags to all the individual sample. There are two ways of doing this step.
   a. Manually assign QC flags through the QC column in Sample Details table.
   b. Automatically assign QC flag by running Assign QC flags automation. This option is for scenarios where a huge number of libraries are involved.

   In the Step Details section, the following step fields are visible. (N is the number of criteria. You can use one or more criteria.)
   - Criteria N - Source Data Field—Select from preset options eg # Reads.
   - Criteria N - Operator—Select from preset options, for example, >= (greater than or equal to).
   - Criteria N - Threshold Value—Enter the desired threshold value.

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3. After filling up the criteria fields, select Assign QC flags. This selection triggers the automation script, which loops through each library in the pool and apply QC flag base on the criteria set previously.
   This automation also generates an AssignQC Result file under Files section.
4. Select Next Steps.
   On the Assign Next Steps screen, the Next Step field for all samples is prepopulated with Mark protocol as complete.
5. Select Finish Step.

At this point, the whole NextSeq 1000/2000 Integration workflow is fully validated.

Troubleshooting

If an automation trigger does not appear to run its corresponding scripts, refer to *Troubleshooting Automation* in the Clarity LIMS API documentation.

If the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3) step starts but does not finish, complete the following steps:

1. Log in to Clarity LIMS using the default user account and use one of the following methods to open the step in Clarity LIMS:
   - Method 1: In Lab View, find the step in the Recent Activities pane.
   - Method 2: Search for the step in Clarity LIMS using reagent cartridge barcode as the search term.
2. On the Record Details screen, locate the Sequencing Log field.
   The multiline text field contains logging information.

If you cannot reach the Record Details screen, or if the Sequencing Log field does not contain enough information to resolve the issue, contact the Clarity LIMS support team. Supply the relevant information from the troubleshooting steps already performed.

NextSeq 1000/2000 Integration v2.4 Release Notes

These Release Notes describe the key changes to software components for the Clarity LIMS NextSeq 1000/2000 Integration Package v2.4.

Compatibility

This version of the integration package is compatible with the following software:

- Clarity LIMS v6.2 and later
- NextSeq 1000/2000 Instrument Control Software (NCS) v1.5.0

New Features

- Updates third-party dependency libraries.

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Defect Repairs

- The NextSeq 1000/2000 sample sheet now reflects the submitted sample name instead of the library name.
- The scope for the JWT token in the integration is restricted.
- Fixed SIS core service not being properly registered in systemctl.

Known Issues

- Sequencing run error triggered by disk space full on instrument causes `RunParameters.xml` to be empty and the run event cannot be processed on Clarity LIMS. This error is unlikely to occur as pre-run checks on the instrument check for sufficient disk space before run is started.

Limitations

For the demultiplexing information in the Sample Details table to display correctly, the following conditions must be met:

- The DRAGEN version must be earlier than v3.9.3.
- The samples in the run must not repeat.
- The run must be for the single lane flow cell (P1 or P2).

NextSeq 1000/2000 Integration v2.3.1 Configuration and User Interaction

The Illumina NextSeq 1000/2000 Integration Package v2.3.1 supports the integration of Clarity LIMS to Illumina NextSeq 1000/2000 sequencing systems.

For instructions on validating and troubleshooting the NextSeq 1000/2000 Integration, refer to *NextSeq 1000/2000 Integration v2.3.1 Validation and Troubleshooting* on page 1559.

> The configuration provided in this integration has been established to support NextSeq 1000/2000 lab processes. Any configuration changes to protocols or workflows—including renaming protocols, steps, and fields—could break the process.

Overview

The integration includes the following features:

- Preconfigured NextSeq 1000/2000 Sequencing v2.3 workflow that maps to lab protocols and instrument runs.
- The following preconfigured protocols:
    - Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3)
    - Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3)
    - AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3)
    - Demultiplexing (NextSeq 1000/2000 Sequencing v2.3)

- Automatic validation of run setup information before sample sheet generation or planned run creation.
- Automated generation of a sample sheet. The sample sheet is used to start the sequencing run on NextSeq 1000/2000 Control Software (NCS) via Local run mode.
- Automated creation of a planned run on Illumina Connected Analytics (ICA). The NCS retrieves and starts the planned run via Hybrid or Cloud run mode.
- Automated tracking of the NextSeq 1000/2000 sequencing run and parsing of run statistics from BaseSpace Sequence Hub into Clarity LIMS using SIS integration service.
- Automated tracking of the secondary analysis using DRAGEN (up to BCL Convert only) and parsing of demultiplexing metrics from BaseSpace Sequence Hub into Clarity LIMS using SIS integration service.
- Unicode `Parameter.xml` file support from NCS v1.5.
- [Optional] Preconfigured Library Prep Validation v2.3.0 workflow used for validation purposes only. The workflow contains a single-step protocol that models the library prep workflow required to produce libraries tagged with index sequences. At the end of the step, these libraries are routed to NextSeq 1000/2000 Sequencing v2.3 workflow. For more information, refer to *NextSeq 1000/2000 Integration v2.3.1 Validation and Troubleshooting* on page 1559.
- [Optional] An integration of Clarity LIMS Product Analytics that make Clarity LIMS data available to the ICA and product analytics services. A subscription to ICA is required for this integration. This integration also requires installation and configuration. For more information, refer to *Clarity LIMS Product Analytics Integration v1.1 Configuration and User Interaction* on page 2415.

Prerequisites and Assumptions

The Illumina NextSeq 1000/2000 Integration Package v2.3 is compatible with Illumina cloud hosted deployments only.

It is assumed that samples entering the NextSeq 1000/2000 Sequencing v2.3 workflow have gone through library preparation and quantification processes. Before they are assigned to the workflow, samples have completed the following actions:

- Samples have been accessioned into Clarity LIMS.
- Samples have been run through QC and library prep.
- Samples have the Molarity (nM) global field set to some value.
  The Calculate Volumes automation in the Library Pooling and Dilution step requires a value in the Molarity (nM) global field.

For more information on sample accessioning, refer to *Sample Accessioning* and *Upload and Modify Samples* in the Getting Started section of the Clarity LIMS Core Functionality documentation.

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You can assign samples to workflows automatically, using a routing script, or manually—from the Projects & Samples dashboard. Refer to *Assign and Process Samples* in the Clarity LIMS Core Functionality documentation.

Workflows, Protocols, and Steps

The NextSeq 1000/2000 Integration Package v2.3.1 includes the following workflows:

- NextSeq 1000/2000 Sequencing v2.3
  Samples are routed to this protocol after they have gone through Library Prep Validation v2.3.0 (or any other library prep workflow). Samples are pooled, diluted to final loading concentration, and added to a reagent cartridge in preparation for the sequencing run.
- [Optional] Library Prep Validation v2.3.0 (recommended for validation purposes).

The workflow configuration includes a global field named Illumina Universal Sample Identifier. This text field is optional and reserved for a future feature. The field value is not required for this integration.The following table lists and describes the protocols and steps included in these workflows.

> The workflow configuration includes a global field named Illumina Universal Sample Identifier. This text field is reserved for Clarity LIMS Product Analytics support. The field is optional and not required for this integration.

Table 320 Illumina NextSeq 1000/2000 Integration Package v2.3.1 Protocols and Steps

Protocol Name	Protocol Purpose	Steps Included in Protocol
Library Prep Validation v2.3.0 Workflow		
Library Prep Validation v2.3.0	Included for validation purposes only, this protocol models the library prep steps required to advance samples to the NextSeq 1000/2000 Sequencing v2.3 workflow. The protocol contains a single step—Library Prep Validation v2.3.0. At the end of this step, a routing script sends the samples to the first step of the NextSeq 1000/2000 Sequencing v2.3 workflow. The first step is Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3).	1. Library Prep Validation v2.3.0
NextSeq 1000/2000 Sequencing v2.3 Workflow		

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Protocol Name	Protocol Purpose	Steps Included in Protocol
NextSeq 1000/2000 Sequencing v2.3	This protocol models the lab processes of starting a NextSeq 1000/2000 sequencing run. Details of the steps: 1. Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3) a. Samples are pooled and diluted to the final loading concentration with the help of the Calculate Volume script. 2. Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3) a. The library pool from step 1 is then ready to be loaded to the NextSeq 1000/2000 reagent cartridge. b. Run and analysis information is validated. c. Sample sheet is generated and/or planned run is created. 3. AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3) a. This step is a fully automated step that is started and completed automatically after the sequencing run is started and completed on the instrument side. b. All the metadata (eg, run configuration, primary run metrics, etc.) of the sequencing run are recorded automatically. Do not add samples to the Ice Bucket or start or complete the step. The integration completes these actions automatically. c. Generated data's secondary analysis is planned by default for the run. Samples continues	1. Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3) 2. Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3) 3. AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3) 4. Demultiplexing (NextSeq 1000/2000 Sequencing v2.3)

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Validation Workflow

The Library Prep Validation v2.3.0 workflow allows for validation of the system after installation is complete. For details, refer to *NextSeq 1000/2000 Integration v2.3.1 Validation and Troubleshooting* on page 1559.

Step Automations

Automation details are provided in the sections following the table.

For details on the validation workflow, refer to *NextSeq 1000/2000 Integration v2.3.1 Validation and Troubleshooting* on page 1559.

Table 321 NextSeq 1000/2000 Sequencing v2.3 Workflow Step Automations

Protocol	Step	Automation
NextSeq 1000/2000 Sequencing v2.3	Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3)	• Calculate Volumes • Register Pools & Register Step Completed[1] • Register Step Started[1] • Set Next Step
	Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3)	• Register Step Completed[1] • Retrieve Analysis Workflow Versions • Set Next Step • Validate Reagent Cartridge Barcode • Validate Run Setup and Create Planned Run • Validate Single Input & Register Step Started[2]
	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3)	• Register NextSeq1k2k RunCompleted & Register Step Completed[1] • Register Step Started & Register NextSeq1k2k Run & Register NextSeq1k2k Run Association & Register NextSeq1k2k RunStarted[1]
	Demultiplexing (NextSeq 1000/2000 Sequencing v2.3)	• Assign Demultiplex QC flags • Register Analysis Completed & Register Step Completed[1] • Register Step Started[1] • Set Next Step

[1] These automations are required for Clarity LIMS Product Analytics support only.
[2] These automations are required for the NextSeq 1000/2000 Sequencing v2.3 workflows and contain additional logic needed for Clarity LIMS Product Analytics support. If you would like to remove Clarity LIMS Product Analytics support, contact Illumina Support.

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Terminology

The Illumina NextSeq 1000/2000 Integration Package v2.3.1 supports Clarity LIMS v5.3 and later.

As of Clarity LIMS v5, the term user-defined field (UDF) has been replaced with custom field in the user interface. However, the API resource is still called UDF.

There are two types of custom fields:

- Master step fields—Configured on master steps. Master step fields only apply to the following:
  - The master step on which the fields are configured.
  - The steps derived from those master steps.
- Global fields—Configured on entities (eg, submitted sample, derived sample, measurement, etc.). Global fields apply to the entire Clarity LIMS system.

Step 1: Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3)

In this step, the addition of RSB dilutes pooled samples. Manually create a working pool based on the final loading concentration required.

Create one pool per step. The Calculate Volumes automation supports one pool only.

Register Step Started

The Register This automation registers the start time of the step by publishing messages to Clarity LIMS Product Analytics through the LINK service. This automation is automatically triggered on entry to the step.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_
started.groovy'
```

Calculate Volumes Automation

This automation is triggered when you select Calculate Volumes on the Record Details screen. The automation completes the following actions:

- Sets RSB Volume for Pool (µl) field value to 24 for calculation purpose of the 2 nM intermediate library pool.
- Copies the Final Loading Concentration (pM) from step inputs to step outputs.

```
-exp 'step.::RSB Volume for Pool (µl):: = 24; output.::Final Loading
Concentration (pM):: = step.::Final Loading Concentration (pM)::'
```

- Calculate per sample volume required for each library to make the 2 nM intermediate library pool (24ul in volume).

```
-exp 'input.::Per Sample Volume (µl):: = ( 48/ input.::Molarity (nM):: );
step.::RSB Volume for Pool (µl):: -= input.::Per Sample Volume (µl)::'
```

- Calculate the volume required of the 2 nM library pool to be diluted further to the Final Loading Concentration (pM) with the Final Loading Volume (µl).

```
-exp 'step.::Library Pool Volume (µl):: = (step.::Final Loading Concentration
(pM):: * step.::Final Loading Volume (µl)::/2000); output.::RSB Volume (µl):: =
step.::Final Loading Volume (µl):: - step.::Library Pool Volume (µl)::'
```

- Uses the `NextSeq1K2K_Pool1.csv`, `NextSeq1K2K_Pool2.csv`, and `NextSeq1K2K_Pool3.csv` template files to generate a single CSV file containing information about the pool and the samples it contains. The generated file is stored in the Calculation File placeholder, in the Files section, for download.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} \
   script:driver_file_generator \
      -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NextSeq1K2K_
      Pool1.csv \
      -o 1.csv \
   script:driver_file_generator \
      -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NextSeq1K2K_
      Pool2.csv \
      -o 2.csv \
   script:driver_file_generator \
      -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NextSeq1K2K_
      Pool3.csv \
      -o 3.csv \
&& cat 1.csv 2.csv 3.csv > {compoundOutputFileLuid0}.csv
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol. The next step is Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3):

```
nextStep = ::ADVANCE::
```

Register Pools & Register Step Completed

This automation is automatically triggered on exit of the step and performs the following tasks:

- Registers the pool and library information used by the Clarity LIMS workflow. Samples in the pool are assumed to have gone through library preparation.
- Registers the completion time of the step by publishing messages to Clarity LIMS Product Analytics through the LINK service.

This automation is only used for Clarity LIMS Product Analytics support.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/pooling.groovy' &&
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_
completed.groovy'
```

Master Step Fields

There are four fields defined on the Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3) step.

Table 322 Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3) Master Step Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Final Loading Concentration (pM)	Numeric Dropdown	Required Field Custom Entries	Presets • 650 • 750 • 1000 • 2000
Final Loading Volume (µl)	Numeric	Required Field	Default • 24

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Library Pool Volume (µl)	Numeric	Read Only	Hidden ⚠ This field is used by the Calculate Volumes automation. Removing, renaming, or modifying the field will break the automation.
RSB Volume for Pool (µl)	Numeric	Read Only	Hidden ⚠ This field is used by the Calculate Volumes automation. Removing, renaming, or modifying the field will break the automation.

Global Fields

The following table lists the global fields that are configured to display on the Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3) step.

Table 323 Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3) Global Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Final Loading Concentration (pM)	Numeric Dropdown	Required Field Custom Entries	Decimal places displayed = 0 Presets • 225 • 400
RSB Volume (µl)	Numeric	Read Only	Decimal places displayed = 2

User Interaction

Refer to *NextSeq 1000/2000 Integration v2.3.1 Validation and Troubleshooting* on page 1559 for details.

Step 2: Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3)

In this step, scan the reagent cartridge barcode into Clarity LIMS, then manually place the working pool into the reagent cartridge for the NextSeq 1000/2000 run. This step validates the run setup and analysis information and generates the sample sheet file and/or creates a planned run on Illumina Connected Analytics, depending on the Run Mode selected.

The NextSeq 1000/2000 reagent cartridges support different read cycle numbers. Make sure that the read cycle values configured for the planned run are within the maximum allowable reads for the cartridge type.

Validate Single Input Automation & Register Step Started Automation

Automatically triggered at the beginning of the step, this automation does the following actions:

- Checks that there is only one container input to the step. This script is required for the NextSeq 1000/2000 Sequencing v2.3 workflow.

```
script:validateSampleCount -min 1 -max 1
```

- Registers the start time of the step by publishing messages to Clarity LIMS Product Analytics through the LINK service. This script is only used for Clarity LIMS Product Analytics support.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar
```

```
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_
started.groovy'
```

Validate Reagent Cartridge Barcode Automation

Automatically triggered on exit of the Placement screen, the following automation validates the reagent cartridge barcode to make sure it conforms to the barcode mask [A-Za-z]{2}[0-9]{7}-[A-Za-z0-9]{4}:

```
if (!output.container.name.matches( ::[A-Za-z]{2}[0-9]{7}-[A-Za-z0-9]{4}:: ) )
{fail ( ::Invalid Reagent Cartridge Barcode. Please verify and try again.:: ) }
```

Retrieve Analysis Workflow Versions Automation

Automatically triggered upon entry of the Record Details screen, this automation completes the following actions:

- Fetches the available analysis workflow versions from ICA.
- Updates the preset values of both Local Analysis Workflow Versions & Cloud Analysis Workflow Versions fields.

```
/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/nextseq1k2k-
automation.jar script:retrieve_analysis_workflow_versions -i {stepURI:v2} -u
{username} -p {password} -l {compoundOutputFileLuid2}
```

Do not modify or disable the Retrieve Analysis Workflow Versions automation script. Modifying or disabling the script breaks the integration.

Validate Run Setup and Create Planned Run Automation

Automatically triggered when a button on the Record Details screen is selected, this automation does the following actions:

- Validates the parameters entered on the Record Details screen. These parameters are used to set up the run and generate the sample sheet file and/or create the planned run on ICA.
  - Run Name can only contain alphanumeric, dash, underscore, or period characters. Spaces are not permitted.

```
if (!step.::Run Name::.matches(::[a-zA-Z0-9-_.]+::))
{ fail(::Run Name contains prohibited characters. Allowed characters are: a-z,
A-Z, 0-9, -, _, and .::) }
```

  - Run Name must not exceed 255 characters.

```
if (step.::Run Name::.length() > 255) { fail(::Run Name shall not exceed 255
characters.::) };
```

- Checks the Index Reads, Index Read 1, and Index Read 2 field values.

  - If Index Reads is No Index, Index Read 1 and Index Read 2 values must be 0 (error results if it is otherwise).
  - If Index Reads is Single Index, Index Read 1 value must be greater than 0, and Index Read 2 values must be 0 (error results if it is otherwise).
  - If Index Reads is Dual Index, Index Read 1 and Index Read 2 values must be greater than 0 (error results if it is otherwise).

```
if (step.::Index Reads:: == ::No Index::)
{ if (step.::Index Read 1:: != 0 || step.::Index Read 2:: != 0) { fail(::Index Read 1 and Index Read 2 must be 0 if the Index Reads is No Index.::) } }
else
{ if (step.::Index Reads:: == ::Single Index::)
{ if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) { fail(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if the Index Reads is Single Index.::) } }
else
{ if (step.::Index Read 1:: == 0 || step.::Index Read 2:: == 0) { fail(::Index Read 1 and Index Read 2 must be greater than 0 if the Index Reads is Dual Index.::) } } }
```

- Checks the Paired End, Read 2 Cycles, and Index Read 2 field values.
  - If Paired End is set to True and Read 2 Cycles value is 0, an error is generated.
  - If Paired End is set to False and Read 2 Cycles or Index Read 2 values are greater than 0, an error is generated.

```
if (step.::Paired End::.toBoolean())
{ if (step.::Read 2 Cycles:: == 0) { fail(::Read 2 Cycles must not be zero if it is Paired End read.::) }}
else
{if (step.::Read 2 Cycles:: != 0 || step.::Index Read 2:: != 0) {fail(::Read 2 Cycles and Index 2 Cycles must be 0 if it is not Paired End Read.:: )}}
```

- Checks the Adapter Sequence Read 1 and Adapter Sequence Read 2 field values.
  - Adapter Sequence Read 1 and Adapter Sequence Read 2 can only contain ACTG+ characters.

```
if (step.hasValue(::Adapter Sequence Read 1::) && !step.::Adapter Sequence Read 1::.matches(::[ACTG+]+::)) { fail(::Adapter Sequence Read 1 contains prohibited characters. Allowed characters are: ACTG+::) };
if (step.hasValue(::Adapter Sequence Read 2::) && !step.::Adapter Sequence Read 2::.matches(::[ACTG+]+::)) { fail(::Adapter Sequence Read 2 contains prohibited characters. Allowed characters are: ACTG+::) }
```

- Checks Override Cycles field value.
  - Override Cycles can only contain Y, N, I, U, 0–9, and semicolon characters.

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```
if (step.hasValue(::Override Cycles::) && !step.::Override Cycles::.matches(::
[YNIU0-9;]+::))
{ fail(::Override Cycles contains prohibited characters. Allowed characters are:
Y, N, I, U, 0-9 and ;. Example: N1Y150;I8;I7N1;Y141U10.::) }
```

- When the Run Mode is Cloud, the script does the following actions:
  - Sets Local Analysis Workflow Versions to None.
  - If the Cloud Analysis Workflow Versions is None, the script displays the following error: `Invalid option selected for Cloud Analysis Workflow Versions field.`

```
if (step.::Run Mode:: == ::Cloud::) { step.::Local Analysis Workflow Versions::
= ::None::; if (step.::Cloud Analysis Workflow Versions:: == ::None::) { fail
(::Invalid option selected for Cloud Analysis Workflow Versions field.::); }};
```

  - Due to automation script limitation, a validation rule resides in the backend service to perform the following action: If Cloud Analysis Workflow Versions is 3.7.4 or later, set FASTQ Compression Format to gzip.
- When the Run Mode is Local or Hybrid, the script does the following actions:
  - Sets Cloud Analysis Workflow Versions to None.
  - If Local Analysis Workflow Versions is None, the script displays the following error message: `Invalid option selected for Local Analysis Workflow Versions field.`

```
if (step.::Run Mode:: == ::Local:: || step.::Run Mode:: == ::Hybrid::) {
step.::Cloud Analysis Workflow Versions:: = ::None::; if (step.::Local Analysis
Workflow Versions:: == ::None::) { fail(::Invalid option selected for Local
Analysis Workflow Versions field.::); }};
```

  - Due to automation script limitation, a validation rule resides in the backend service. If Local Analysis Workflow Versions is 3.7.4 or later, an error occurs if FASTQ Compression Format is None.
- Generates the sample sheet and/or creates the planned run on ICA.
  - Sample sheet is attached to the step.

```
/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/nextseq1k2k-
automation.jar
script:generate_v2_sample_sheet -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid2} -c {compoundOutputFileLuid0}
```

  - There is backend validation of the sample sheet content. This validation makes sure that the sample sheet is valid to set up a Local Mode run or is ready for DRAGEN applications. Refer to *NextSeq 1000/2000 Integration v2.3.1 Validation and Troubleshooting* on page 1559 for submitted sample name character restrictions.

Set Next Step Automation

Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol—AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3). The automation is automatically triggered on exit of the Record Details screen.

```
nextStep = ::ADVANCE::
```

Register Step Completed

Automatically triggered on exit of the step, this automation registers the completion time of the step by publishing messages to Clarity LIMS Product Analytics through the LINK service. This script is only used for Clarity LIMS Product Analytics support.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_completed.groovy'
```

Master Step Fields

There are 24 fields defined on the Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3) step. These fields are required for sample sheet generation and planned run creation on ICA.

Table 324 Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3) Master Step Fields Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Run Name	Text	Required Field	
Instrument Type	Text Dropdown	Required Field	Presets • NextSeq1000 • NextSeq2000
Run Mode	Text Dropdown	Required Field	Presets • Local • Hybrid • Cloud
Paired End	Text Dropdown	Required Field	Presets • True • False

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Read 1 Cycles	Numeric Dropdown	Required Field Custom Entries	Presets • 301 • 151 • 101 • 51
Read 2 Cycles	Numeric Dropdown	Required Field Custom Entries	Presets • 301 • 151 • 101 • 51
Index Reads	Text Dropdown	Required Field	Presets • No Index • Single Index • Dual Index
Index Read 1	Numeric Dropdown	Required Field Custom Entries	Presets • 0 • 6 • 8
Index Read 2	Numeric Dropdown	Required Field Custom Entries	Presets • 0 • 6 • 8
Analysis Workflow	Text	Required Field Read Only	Default • GenerateFASTQ
Adapter Sequence Read 1	Text		
Adapter Sequence Read 2	Text		
Barcode Mismatches Index 1	Numeric Dropdown		Presets • 0 • 1 • 2

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Barcode Mismatches Index 2	Numeric Dropdown		Presets • 0 • 1 • 2
Override Cycles	Text		
Cloud Run ID	Text	Read Only	Hidden - Used in creating planned run in ICA. Do not delete, rename, or modify. These actions will break the integration.
Use Custom Index Read 1 Primer	Toggle Switch		Hidden Custom primer option is currently not supported. Do not enable as it will break the integration.
Use Custom Index Read 2 Primer	Toggle Switch		Hidden Custom primer option is currently not supported. Do not enable as it will break the integration.

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Use Custom Read 1 Primer	Toggle Switch		Hidden Custom primer option is currently not supported. Do not enable as it will break the integration.
Use Custom Read 2 Primer	Toggle Switch		Hidden Custom primer option is currently not supported. Do not enable as it will break the integration.
Instructions	Multi-Line Text	Read Only	Default • If GenerateFASTQ analysis is required, please select the DRAGEN version in Cloud Analysis Workflow Versions (for Cloud run mode) or Local Analysis Workflow Versions (for Local/Hybrid run mode) custom fields above.
Local Analysis Workflow Versions	Text Dropdown		Default • None

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Cloud Analysis Workflow Versions	Text Dropdown		Default • None
FASTQ Compression Format	Text Dropdown	Required Field	Presets • gzip (default value) • DRAGEN • None

Do not modify the configuration of the Local Analysis Workflow Versions and Cloud Analysis Workflow Versions fields. Modifying these fields can break the integration.

User Interaction

Refer to *NextSeq 1000/2000 Integration v2.3.1 Validation and Troubleshooting* on page 1559 for details.

Step 3: AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3)

This step is fully automated.

The integration starts and completes the step automatically. Data from the run is parsed back to Clarity LIMS. No user interaction is required. In this step, the pooled samples in the reagent cartridge are sequenced on the NextSeq 1000/2000 instrument.

Do not add samples to the Ice Bucket or start or complete the step. The integration completes these actions automatically.

Register Step Started & Register NextSeq1k2k Run & Register NextSeq1k2k Run Association & Register NextSeq1k2k RunStarted

Automatically triggered at the beginning of the step, this automation completes the following actions:

- Registers the start time of the step.
- Registers the NextSeq 1000/2000 sequencing run configuration.
- Registers the association of the sequencing run with the actual samples by linking the Instrument Run ID with the IDs for the samples.
- Registers the time that the sequencing run starts.

The automation completes these actions by publishing messages to Clarity LIMS Product Analytics through the LINK service. This automation is only used for Clarity LIMS Product Analytics support.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/common/step_
started.groovy' && /opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/unified-product-analytics/automation/unified-
product-analytics-automation.jar script:executeUPAAutomationScript -i
{stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0} -s
'com/illumina/upa/scripts/nextseq1k2k/register_nextseq1k2k_run.groovy' &&
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/nextseq1k2k/nextseq1k2k_
associate_seqrun.groovy' && /opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/unified-product-analytics/automation/unified-
product-analytics-automation.jar script:executeUPAAutomationScript -i
{stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0} -s
'com/illumina/upa/scripts/nextseq1k2k/nextseq1k2k_seqrun_started.groovy'
```

Register NextSeq1k2k RunCompleted & Register Step Completed

Automatically triggered on exit of the step, this automation completes the following actions:

- Registers the sequencing run completion time, run status, and run metrics. This script requires the Cloud Run ID field from the Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3) step.
- Registers the completion time of the step.

The automation completes these actions by publishing messages to Clarity LIMS Product Analytics through the LINK service. This automation is only used for Clarity LIMS Product Analytics support.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/nextseq1k2k/nextseq1k2k_
seqrun_completed.groovy' && /opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/unified-product-analytics/automation/unified-
product-analytics-automation.jar script:executeUPAAutomationScript -i
{stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0} -s
'com/illumina/upa/scripts/common/step_completed.groovy'
```

Master Step Fields

There following fields are defined on the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3) step. These fields are used to display the run status and sequencing run and analysis configuration parsed from the `RunParameters.xml` file of the sequencing run.

Do not modify the master step field names. This action causes the integration to break.

Run Parameters and Corresponding Clarity LIMS Step Fields

The following table shows how some of the step fields map to the fields on the `RunParameters.xml` file, and whether the field is visible on the Record Details screen.

Master Step Field	RunParameters.xml Field	On Record Details Screen
Current Cycle	Calculated based on CompletedCycles Field	Visible
Current Read	Calculated based on CompletedCycles field against PlannedCycles	Visible
Flow Cell ID	FlowCellSerialNumber	Visible
Flow Cell Lot Number	FlowCellLotNumber	Visible
Instrument Control Software Version	ApplicationVersion	Visible
Instrument ID	InstrumentSerialNumber	Visible
Output Folder	OutputFolder	Visible
Reagent Cartridge ID	CartridgeSerialNumber	Visible
Reagent Cartridge Lot Number	CartridgeLotNumber	Visible
RTA Version	RtaVersion	Visible
Run Name	ExperimentName	Visible
Secondary Analysis Workflow	SecondaryAnalysisWorkflow	Visible

Master Step Field	RunParameters.xml Field	On Record Details Screen
Flow Cell Mode	FlowCellMode	Hidden Do not delete, rename, or modify. These actions can break the integration.
Instrument Run ID	Derived from OutputFolder	Hidden Do not delete, rename, or modify. These actions can break the integration.
Run End Time	RunEndTime	Hidden Do not delete, rename, or modify. These actions can break the integration.
Run Start Time	RunStartTime	Hidden Do not delete, rename, or modify. These actions can break the integration.
Secondary Analysis Mode	SecondaryAnalysisMode	Hidden Do not delete, rename, or modify. These actions can break the integration.
Secondary Analysis Platform Version	SecondaryAnalysisPlatformVersion	Hidden Do not delete, rename, or modify. These actions can break the integration.

Master Step Field	RunParameters.xml Field	On Record Details Screen
SkipObdd	SkipObdd	Hidden Do not delete, rename, or modify. These actions can break the integration.

Additional Master Step Fields and Values

The following table shows how the other step fields derive their values, and whether the step field is visible on the Record Details screen.

Master Step Field	RunParameters.xml Field	On Record Details Screen
Instrument Platform	NextSeq 1000/2000 Constant value	Visible
Instrument Type	One of the following options: • NextSeq1000 • NextSeq2000 Determined from Instrument Type step field from previous Load to Reagent Cartridge step.	Visible
Run Status	One of the following options: • RunStarted • RunCompletedSuccessfully • RunAbortedByUser • RunErroredOut Set by the SIS integration service[1]	Visible
Sequencing Log	Filled in by the SIS integration service[1] as the sequencing run proceeds	Visible
BaseSpace Run ID	Received from SQS message in the SIS integration service[1]	Hidden Do not delete, rename, or modify. These actions will break the integration.

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[1]For information on how the integration works, refer to *Run Status, Primary Metrics, and Analysis Results Parsing and Recording* in *How the NextSeq 1000/2000 Integration Works* on page 1550.

Global Fields

The following global fields are used to capture the run metrics in Clarity LIMS.

- % Bases >=Q30 R1
- % Bases >=Q30 R2
- % Error Rate R1
- % Error Rate R2
- Yield (Gb) R1
- Yield (Gb) R2
- Reads PF R1
- Reads PF R2
- %PF R1
- %PF R2
- % Aligned R1
- % Aligned R2
- % Phasing R1
- % Phasing R2
- % PrePhasing R1
- % PrePhasing R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- Cluster Density R1
- Cluster Density R2

At the end of this step, the pool of samples is automatically advanced to (and queued for) the Demultiplexing (NextSeq 1000/2000 Sequencing v2.3) step.

Step 4: Demultiplexing (NextSeq 1000/2000 Sequencing v2.3)

This step is a semi-automated step.

The integration starts the step automatically and demultiplexing data from the GenerateFASTQ secondary analysis is parsed back to Clarity LIMS. The lab scientist reviews the demultiplexing result parsed into Clarity LIMS, assigns QC flags, and completes the step.

For the demultiplexing information in the Sample Details table to display correctly, the following conditions must be met:

- The DRAGEN version must be earlier than v3.9.3.
- The samples in the run must not repeat.
- The run must be for the single lane flow cell (P1 or P2).

Do not add samples to the Ice Bucket or start or complete the step. The integration completes these actions automatically.

Register Step Started Automation

Automatically triggered at the beginning of the step, this automation registers the start time of the step by publishing messages to Clarity LIMS Product Analytics through the LINK service. This automation is only used for Clarity LIMS Product Analytics support.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_started.groovy'
```

Assign Demultiplexing QC Flags Automation

Automatically triggered when you select a button on the Record Details screen, this automation assigns QC flags based on the criteria set in the step fields.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -u {username} -p {password} script:assignQCForDemuxStep -i {processURI:v2} -log {compoundOutputFileLuid1} -qcResult {compoundOutputFileLuid2}
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation sets the next step to Advance and the samples to complete the protocol.

```
nextStep = ::ADVANCE::
```

Register Analysis Completed & Register Step Completed Automation

Automatically triggered on exit of the step, this automation performs the following actions:

- Registers the NextSeq 1000/2000 analysis status and result.
- Registers the completion time of the step.

These actions are done by publishing messages to Clarity LIMS Product Analytics through the LINK service. This automation is only used for Clarity LIMS Product Analytics support.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_completed.groovy' && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/nextseq1k2k/nextseq1k2k_analysisrun_completed.groovy'
```

Master Step Fields

The following table lists the 12 master step fields that are configured on the Demultiplexing (NextSeq 1000/2000 Sequencing v2.3) master step.

Table 325 Master Step Field Configuration for Demultiplexing (NextSeq 1000/2000 Sequencing v2.3) Step

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Criteria 1 - Source Data Field	Text Dropdown	Custom Entries	Presets • # Reads • # Perfect Index Reads • # One Mismatch Index Reads • # of >= Q30 Bases (PF)
Criteria 2 - Source Data Field	Text Dropdown	Custom Entries	Presets • # Reads • # Perfect Index Reads • # One Mismatch Index Reads • # of >= Q30 Bases (PF)

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Criteria 1 - Operator	Text Dropdown	Custom Entries	Presets • >= • <= • = • !=
Criteria 2 - Operator	Text Dropdown	Custom Entries	Presets • >= • <= • = • !=
Criteria 1 - Threshold Value	Numeric		Valid integer value
Criteria 2 - Threshold Value	Numeric		Valid integer value
Log	Multiline Text	Read Only	
Analysis Completed Time	Text	Read Only	Hidden Do not delete, rename, or modify. These actions will break the integration.
Analysis Started Time	Text	Read Only	Hidden Do not delete, rename, or modify. These actions will break the integration.

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Analysis Status	Text	Read Only	Hidden Do not delete, rename, or modify. These actions will break the integration.
BSSH Analysis ID	Text	Read Only	Hidden Do not delete, rename, or modify. These actions will break the integration.
Reagent Cartridge ID	Text	Read Only	Hidden Used for parsing demultiplexing results Do not delete, rename, or modify. These actions will break the integration.

Global Fields

The following table lists the global fields that are configured on the Demultiplexing (NextSeq 1000/2000 Sequencing v2.3) step. These fields are used to display the demultiplexing result metrics for individual library in the sample pool.

Table 326 Global Field Configuration for Demultiplexing (NextSeq 1000/2000 Sequencing v2.3) Step

Field Name	Field Type	Field Constraints/Options
# of >= Q30 Bases (PF)	Numeric	Read Only

Field Name	Field Type	Field Constraints/Options
# One Mismatch Index Reads	Numeric	Read Only
# Perfect Index Reads	Numeric	Read Only
# Reads	Numeric	Read Only
Mean Quality Score (PF)	Numeric	Read Only

User Interaction

Refer to *NextSeq 1000/2000 Integration v2.3.1 Validation and Troubleshooting* on page 1559 for details.

How the NextSeq 1000/2000 Integration Works

Sample Sheet Generation and Planned Run Creation

On the Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3) step, when the Validate Run Setup and Create Planned Run automation is triggered, the run and analysis parameters entered in the Run Details screen are sent to Illumina Connected Analytics (ICA). ICA validates the run and analysis configuration, and sends an error message to Clarity LIMS if the validation fails. If the validation passes, ICA generates the sample sheet (and sends back to Clarity LIMS) and/or creates a planned run depending on the Run Mode selected.

- Local run mode: Only the sample sheet is generated and stored on Clarity LIMS. The sample sheet contains all the run and analysis configuration required to start the run on the instrument.
- Hybrid/Cloud run mode: A planned run is created on ICA. This planned run contains all the run and analysis configuration required to start the run on the instrument. The sample sheet is also generated and stored on Clarity LIMS for reference purposes.

Refer to *Start A Sequencing Run On Instrument With Different Run Modes* on page 1556 for details on how to start a run using different run mode.

Run Status, Primary Metrics, and Analysis Results Parsing and Recording

After the sequencing run is started on the instrument, NextSeq 1000/2000 Control Software (NCS) notifies BaseSpace Sequence Hub that the sequencing run has started.

BSSH then does the following actions:

- Local run mode: Creates a run on the BaseSpace Sequence Hub database with the run configuration information received from NCS.
- Hybrid/Cloud run mode: Creates a run on the BaseSpace Sequence Hub database by copying the run configuration information from the planned run created in ICA, using a special Cloud Run ID received from NCS.

After the run is created on BaseSpace Sequence Hub, a run update event message that carries the RunStarted equivalent message is created on BaseSpace Sequence Hub and delivered through ICA and, eventually, to Clarity LIMS SQS queue service. The SIS integration service captures and processes the message.

Based on the reagent cartridge barcode information in the event message (refer to the following example), SIS looks in the queue of AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3) step for a container with the same reagent cartridge barcode. When identified, the step is started automatically and the Run Status field on Record Details screen is updated to RunStarted. SIS then updates all the other step fields (eg, Current Read, Output Folder) by parsing the details from the `RunParameters.xml` uploaded to BaseSpace Sequence Hub by NCS.

```
#Example of an run update event
{
"acl": [
"wid:61dc14e8-d823-3bd1-bea3-66f9c8388254"
],
"apiUrl": "https://api.basespace.illumina.com/v2/runs/r.ZvTfDXsNhUyh2kA5S4Zewg",
"dateModified": "2020-03-26T09:19:58.5546497Z",
"flowcellBarcode": "11M11COMC",
"gdsFolderPath": "/Runs/200326_MP2-15_87_11M11COMC_r.ZvTfDXsNhUyh2kA5S4Zewg",
"gdsVolumeName": "bssh.61dc14e8d8233bd1bea366f9c8388254",
"id": "r.ZvTfDXsNhUyh2kA5S4Zewg",
"instrumentRunId": "200326_MP2-15_87_11M11COMC",
"name": "200326_MP2-15_87_11M11COMC",
"reagentBarcode": "EC0001990-EC5",
"sampleSheetName": "SampleSheet.csv",
"status": "Running",
"v1pre3Id": "194424236"
}
```

How the SIS Integration Service Handles Run Status

The NCS continues to notify BaseSpace Sequence Hub of any run status change throughout the course of the sequencing run. The run update event delivery process continues as described in the previous section.

The following table lists the run status details and how the SIS integration service handles each status.

Run Status	Shown in Step	Details
RunStarted	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3)	Run Status field is updated to RunStarted. All other step fields (except Run End Time) are updated based on `RunParameters.xml` detail obtained from BaseSpace Sequence Hub, the SQS event message, and the step field value from the Load to Reagent Cartridge (NextSeq 1000/2000 v2.3) step. Reagent cartridge lot information received from BaseSpace Sequence Hub is compared against the details of the pre-accessioned reagent cartridge on Clarity LIMS. • If the expiration date is different, it is updated to the date received from BaseSpace Sequence Hub. • If reagent cartridge is not found, it is created and set to Active. • If reagent cartridge is not in Active status, it is set to Active. Multiline Sequencing Log field is updated.

Run Status	Shown in Step	Details
RunCompletedSuccessfully	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3)	Run Status field is updated to RunCompletedSuccessfully Current Read and Current Cycle fields are updated to the final cycle number and read number based on `RunParameters.xml` detail obtained from BaseSpace Sequence Hub. Run End Time field is updated based on RunParameters.xml detail obtained from BaseSpace Sequence Hub. Sequencing run result (primary metrics) is downloaded from BaseSpace Sequence Hub and the fields in Sample Details table (eg, % Error Rate R1, % Phasing, etc.) are updated with the values. Multiline Sequencing Log field is updated. SIS autocompletes the step and routes the samples in the reagent cartridge container to the next step (eg, Demultiplexing (NextSeq 1000/2000 Sequencing v2.3)) step.

Run Status	Shown in Step	Details
RunErroredOut	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3)	Run Status field is updated to RunErroredOut. Current Read and Current Cycle fields are updated to the final cycle number and read number that the run stopped based on `RunParameters.xml` detail obtained from BaseSpace Sequence Hub. Run End Time field is updated based on `RunParameters.xml` detail obtained from BaseSpace Sequence Hub. Sequencing run result (primary metrics) is downloaded from BaseSpace Sequence Hub and the fields in Sample Details table (eg, % Error Rate R1, % Phasing, etc.) are updated with the values. Multiline Sequencing Log field is updated. SIS does not autocomplete the step. Complete the step manually.

Run Status	Shown in Step	Details
RunAbortedByUser	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3)	Run Status field is updated to RunAbortedByUser. Current Read and Current Cycle fields are updated to the final cycle number and read number that was aborted based on `RunParameters.xml` details obtained from BaseSpace Sequence Hub. Run End Time field is updated based on RunParameters.xml detail obtained from BaseSpace Sequence Hub. Sequencing run result (primary metrics) is downloaded from BaseSpace Sequence Hub and the fields in Sample Details table (eg, % Error Rate R1, % Phasing, etc.) are updated with the values. Multiline Sequencing Log field is updated. SIS does not autocomplete the step. Complete the step manually.
AnalysisStarted	Demultiplexing (NextSeq 1000/2000 Sequencing v2.3)	SIS starts the step automatically for the reagent cartridge container. Multiline Sequencing Log field is updated.

Run Status	Shown in Step	Details
AnalysisCompleted	Demultiplexing (NextSeq 1000/2000 Sequencing v2.3)	SIS downloads the `Demultiplex_stats.csv` file, which contains the demultiplexing result from BaseSpace Sequence Hub. The file is parsed and details are recorded in the Sample Details table for each library in the library pool. Hidden master step fields are updated with information from BaseSpace Sequence Hub. In particular, Analysis Status is updated to Complete. Multiline Sequencing Log field is updated. SIS does not autocomplete the step. Assign the QC flag to the individual library and manually complete the step.
AnalysisFailed	Demultiplexing (NextSeq 1000/2000 Sequencing v2.3)	Hidden step fields are updated with information from BaseSpace Sequence Hub. In particular, Analysis Status is updated to either TimedOut or Aborted. Multiline Sequencing Log field is updated. SIS does not auto-complete the step. Assign the QC flag to the individual library and manually complete the step.

Start A Sequencing Run On Instrument With Different Run Modes

Local Run Mode

1. On the NextSeq 1000/2000 Control Software (NCS) interface, select the menu button and select Settings.
2. Select Local Run Setup and Proactive, Run Monitoring and Storage*.
3. [Optional] Enter the default output folder location.
4. Select Save.
5. On the main screen, select Start.
6. Sign in to the BaseSpace Sequence Hub (BSSH) account that was used during NextSeq 1000/2000 Integration package installation setup.

a. Select the workgroup (if applicable).
b. Select Next.

7. Select Choose, and a file browser pops up.
8. Browse and select the sample sheet generated during the Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3) step.
   When selected, the sample sheet has its content validated. After the validation passes, run and analysis configuration is parsed and autopopulated in the next page.
9. Select Review to review the sequencing run settings.
10. Select Load and manually load the reagent cartridge and flow cell.
11. Select Sequence and the pre-run check proceeds.
    After the pre-run check passes, the sequencing run starts automatically.

Hybrid or Cloud Run Mode

1. On the NextSeq 1000/2000 Control Software interface, select the menu button and select Settings.
2. Select Online Run Setup and Proactive, Run Monitoring and Storage*.
3. [Optional] Enter the default output folder location.
4. Select Save.
5. On the main screen, select Start.
6. Sign into the BSSH account that was used during NextSeq 1000/2000 Integration package installation setup.
   a. Select the workgroup (if applicable).
   b. Select Next.
      Planned runs that were created in the Load to Reagent Cartridge (NextSeq 1000/2000 v2.3) step using the Hybrid or Cloud run modes are displayed.
7. Select the run and select Review to review the sequencing run settings.
8. Select Load and manually load the reagent cartridge and flow cell.
9. Select Sequence and the pre-run check proceeds.
   After the pre-run check passes, the sequencing run starts automatically.

Components Installed

The following sections describe the various components that are installed by default as part of this integration. These components include the following items:

- Files
- Properties
- Reagent categories/label groups
- Reagent kits

- Control types
- Containers

Information on installed workflows, protocols, steps, and automation points is provided in the Workflows, Protocols, and Steps section of *NextSeq 1000/2000 Integration v2.3.1 Configuration and User Interaction* on page 1523.

Reagent Categories/Label Groups

- TruSeq HT adapters v2 (D7-D5)

Reagent Kits

- Resuspension Buffer (RSB)
- NextSeq 1000/2000 reagent cartridge

Container Types

- Tube
- 96-well plate
- NextSeq 1000/2000 reagent cartridge

Control Types

- PhiX v3

This integration supports the NextSeq 1000/2000 reagent cartridge with barcode provided in the format [A-Za-z]{2}[0-9]{7}-[A-Za-z0-9]{4} (eg, EC1234567-EC03).

Rules and Constraints

- The workflow configuration contains several validation checks. To make sure that the calculations work properly, it is important that you do not disable any of this validation logic. The validation checks determine:
  - Which samples, and how many, can enter each step together.
  - Which samples, and how many, can be pooled together.
- The NextSeq 1000/2000 reagent cartridge barcode must be unique. There should not be multiple NextSeq 1000/2000 reagent cartridge containers in the system with the same name.
- Reagent labels (indexes) must be unique.
- One library pool can only contain one library or control with no label (index).

- Do not manually start or complete the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3) step. This step is a fully automated step, and the SIS integration service does not update samples correctly if they have been manually started.
- Do not manually start the Demultiplexing (NextSeq 1000/2000 Sequencing v2.3) step. This step is semi-automated, and the SIS integration service does not update the demultiplexing result correctly if they have been manually started.
- For the automated run to start successfully, select Validate Run Setup and Create Planned Run in the Load to Reagent Cartridge step.

For integrations that run on Clarity LIMS v5.4, the events in the RECENT ACTIVITIES panel can reflect as the API access user instead of the user who ran the step. This is fixed in Clarity LIMS v6.0.

NextSeq 1000/2000 Integration v2.3.1 Validation and Troubleshooting

This section explains how to validate the installation of the Illumina NextSeq 1000/2000 Integration Package v2.3.1.

The validation process involves the following items:

- Running samples through the Library Prep Validation v2.3.0 workflow.
  - The workflow contains a single-step protocol that models the library prep workflow required to produce libraries tagged with index sequences. At the end of the step, these libraries are routed to NextSeq 1000/2000 Sequencing v2.3 workflow.
- Running the libraries through the NextSeq 1000/2000 Sequencing v2.3 workflow. This process validates the following information:
  - Successful sequential step advancement of samples in the following steps:
    1. Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3)
    2. Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3)
    3. AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3)
    4. Demultiplexing (NextSeq 1000/2000 Sequencing v2.3)
  - Automatic validation of run setup information before sample sheet generation or planned run creation.
  - Automated generation of a sample sheet. The sample sheet is used to start the sequencing run on NextSeq 1000/2000 Control Software (NCS) via Local run mode.
  - Automated creation of a planned run on Illumina Connected Analytics. The NextSeq 1000/2000 Control Software retrieves the planned run to start the run via Hybrid or Cloud run mode.
  - Automated tracking of the NextSeq 1000/2000 sequencing run and parsing of run statistics from BaseSpace Sequence Hub into Clarity LIMS using the SIS Integration Service.
  - Automated tracking of the secondary analysis using DRAGEN (up to BCL Convert only).

- Parsing of demultiplexing metrics from BaseSpace Sequence Hub into Clarity LIMS using SIS integration service.

Before running the validation steps below, these steps assume that the NextSeq 1000/2000 Integration Package v2.3.1 is installed, and that you have imported the default Clarity LIMS configuration.

For information on how the integration works, refer to *NextSeq 1000/2000 Integration v2.3.1 Configuration and User Interaction* on page 1523.

Activate Workflow, Create Project, Add and Assign Samples

The following steps set up Clarity LIMS in preparation for running samples through the Library Prep Validation v2.3.0 and NextSeq 1000/2000 Sequencing v2.3 workflows.

1. On the Configuration tab, under Workflows, activate both the Library Prep Validation v2.3.0 and NextSeq 1000/2000 Sequencing v2.3 workflows.
2. On the Projects and Samples screen, create a project and add samples to it.

   Use only alphanumeric, dash, and underscore characters in the submitted sample names. Failing to do so causes sample sheet validation failure in Load To Reagent Cartridge step.

3. Assign the samples to the Library Prep Validation v2.3.0 workflow.

Library Prep Protocol: Library Prep Validation v2.3.0

This single-step protocol models the library prep required to produce libraries tagged with index sequences that are ready for the NextSeq 1000/2000 Sequencing v2.3 workflow.

Run Library Prep Validation v2.3.0 Step

1. In Lab View, locate the Library Prep Validation v2.3.0 protocol.
   Samples are queued for the Library Prep Validation v2.3.0 step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select the label groups to use.
   - TruSeq HT Adapters v2 (D7-D5) is the default label group delivered by Library Prep Validation v2.3.0 protocol.
   - For other reagent labels (indexes), manually create them in the Configuration screen under Consumables > Labels.

     For more information on how to create reagent label group, refer to the *Add and Configure Labels and Label Groups* section in the *Clarity LIMS Core Functionality* documentation.

4. Select Begin Work.
5. On the Placement screen, performing the following actions:
   a. In the Placed Samples area in the Container field, scan or enter the barcode of the destination container.

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b. Select the samples from the Samples to be Placed area, then drag them over to the container wells on the right.

c. Select Add Labels.

6. On the Add Labels screen, the Labels to Add list is populated with labels from the TruSeq HT Adapters v2 (D7-D5) label group. This label group is the only one configured in the out-of-the-box integration. It is used by default in this step.

   a. Select labels on the left, and drag them over to the container on the right to assign them to placed samples.

   b. Select Record Details.

7. On the Record Details screen, perform the following actions:

   a. In the Sample Details table, enter values for Molarity (nM) for all the individual sample. Enter values individually or use the copy/paste functionality to enter multiple values from a spreadsheet.

      - Molarity (nM) values are used in Calculate Volume automation script in Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3) step.

        If Molarity (nM) values are not entered, the Calculate Volume automation script does not work properly.

   b. The Sequencing Instrument column indicates the instrument to be used for the run. Select NextSeq 1000/2000.

   c. Populate the fields in this column using one of the following methods:

      - To populate fields individually for each sample, select from the drop-down list in each row.
      - To populate fields all at one time, use the Copy and Paste buttons to enter multiple values from a spreadsheet (refer to the following note).

      Alternatively, select inside the table and press Ctrl + A to select all rows. At the top of the table, select Sequencing Instrument, then select NextSeq 1000/2000 from the adjacent drop-down list. Select Apply.

      If working with many samples, copy and paste to insert multiple values from a spreadsheet as follows.

      a. Select the Copy button to copy the sample information from Clarity LIMS to the clipboard.

      b. Open a blank Excel document and paste the contents of the clipboard into the spreadsheet.

      c. In the spreadsheet, enter the Molarity (nM) and Sequencing Instrument values. Select the entire spreadsheet, including the headers, (Ctrl + A) and copy (Ctrl + C) the information to the clipboard.

      d. In Clarity LIMS, select the Paste button. Paste the contents of the clipboard (Ctrl + V) into the Update Table window and then select Update to paste into the Sample Details table.

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8. Select Next Steps.
   This selection triggers the Next Steps automation, which sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step successfully.
9. On the Assign Next Steps screen in the Samples table, the Next Step for all samples is prepopulated with Remove from workflow.

   > Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.

10. Select Finish Step.
    On exit from the step, the Routing Script automation is triggered. This automation assigns samples to the first step of the NextSeq 1000/2000 Sequencing v2.3 workflow, which is Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3) step.

NextSeq 1000/2000 Sequencing v2.3 Protocol

Step 1: Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3)

1. In Lab View, locate the NextSeq 1000/2000 Sequencing v2.3 protocol. The samples are queued for the Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Pooling screen, perform the following actions:
   a. Create a pool by dragging samples into the Pool Creator.

      > Do not create more than one pool for this step. The Calculate Volumes automation in this step supports one pool only.

   b. Type a name for the pool or accept the default name (Pool #1).
   c. Select Record Details.
      On the Record Details screen, the Reagent Lot Tracking section tracks the Resuspension Buffer (RSB) lot information used in the step.
5. To add and activate additional reagent lots, perform the following steps:
   a. In a new browser tab, open an additional instance of Clarity LIMS.
   b. Navigate to the Consumables configuration screen.
   c. Select the RSB reagent kit and select New Lot.
   d. Enter the lot details;
   e. For Status of Reagent Lot, select Active.
   f. Select Save.

6. In the Reagent Lot Tracking section, select from the active lots displayed in the drop-down list.
   The Step Details area contains two required fields:
   - Final Loading Concentration (pM)—The value entered in this field is the recommended final loading concentration specified in the *Illumina NextSeq 1000/2000 Sequencing System Guide* at `support.illumina.com`. The value depends on the library type. The default drop-down list contains the values 650, 750, 1000, and 2000. A custom value is acceptable.
   - Final Loading Volume (µl)—The value in this field is the final loading volume of the pool into the reagent cartridge. The field is prepopulated with the configured default value, 24 µl, specified in the *Illumina NextSeq 1000/2000 Sequencing System Guide* at `support.illumina.com`. The value is editable when more volume is necessary.
7. Select Calculate Volumes.
   This selection triggers the Calculate Volumes automation. This automation calculates the volume required for each library to form a pool that has the concentration and volume specified in the step details fields.
   The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains volume information of each of the samples and RSB buffer to add to the pool. Select the file to download it, then open it in Excel.
8. In the Sample Details table, select the pool icon to view details on the pool composition.
9. Select Next Steps.
   This selection triggers the Set Next Step automation, which sets the next step for samples to ADVANCE, advancing them to the next step in the protocol. The next step is Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3).
   On the Assign Next Steps screen, the next step for samples is already set to the next step in the workflow. The next step is Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3).
10. Select Finish Step.

At the end of this step, the pool of samples automatically advances to Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3) step.

Step 2: Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3)

1. In Lab View, locate the NextSeq 1000/2000 Sequencing v2.3 protocol. The pool of samples is queued for the Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
   The Validate Single Input automation is triggered. This automation checks that there is only one container input to the step.
4. On the Placement screen, perform the following actions:
   a. Drag the pool into the NextSeq 1000/2000 Reagent Cartridge field in the Placed Samples area.

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b. Scan or type the barcode of the reagent cartridge into the NextSeq 1000/2000 Reagent Cartridge field.

c. Select Record Details.

On exit of the Placement screen, the Validate Reagent Cartridge Barcode automation checks that the reagent cartridge barcode conforms to the barcode mask [A-Za-z]{2}[0–9]{7}-[A-Za-z0-9]{4}. If not, an error message displays.

On entry to the Record Details screen, the Retrieve Analysis Workflow Versions automation fetches the available analysis workflow versions from ICA. It then updates the preset values of both Local Analysis Workflow Versions & Cloud Analysis Workflow Versions field.

5. On the Record Details screen, the Reagent Lot Tracking section tracks the NextSeq 1000/2000 Reagent Cartridge lot information used in the step. Follow the steps in Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3) step if you must add a lot.
6. In the Reagent Lot Tracking section, select from the active lots displayed in the drop-down list.
7. The fields displayed on the Record Details screen are used to create planned run and generate the sample sheet file.
   - Run Name—Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.
   - Instrument Type—Select from preset options (NextSeq 1000 or NextSeq 2000).
   - Run Mode—Select from preset options: Local, Hybrid, Cloud. For more information on the different run modes, refer to *Run Mode Details* on page 1565.
   - Paired End—Select from preset options (True or False).
   - Read 1 Cycles—Select from preset options (301, 151, 101, 51) or type a custom value.
   - Read 2 Cycles—Select from preset options (301, 151, 101, 51) or type a custom value.
   - Index Reads—Select from preset options (No Index, Single Index, Dual Index).
   - Index Read 1—Select from preset options (0, 6, 8) or type a custom value.
   - Index Read 2—Select from preset options (0, 6, 8) or type a custom value.
   - Analysis Workflow—Read only. This field is set to GenerateFASTQ.
   - Local Analysis Workflow Versions—[Optional] (default is None). Only mandatory when Run Mode = Local/Hybrid and Analysis Workflow = GenerateFASTQ. Select according to the DRAGEN version installed on the instrument.
   - Cloud Analysis Workflow Versions—[Optional] (default is None). Only mandatory when Run Mode = Cloud and Analysis Workflow = GenerateFASTQ. Select the cloud DRAGEN version to be used.
   - FASTQ Compression Format:
     - If Run Mode = Local/Hybrid, Analysis Workflow = GenerateFASTQ, and Local Analysis Workflow Version >= 3.7.4, select gzip or DRAGEN.

    - If Run Mode = Cloud, Analysis Workflow = GenerateFASTQ, and Local Analysis Workflow Version >= 3.7.4, field is set to gzip automatically.
    - Otherwise, select None.
- Adapter Sequence Read 1—[Optional] Enter the Read 1 adapter sequence of the index adapter kit.
- Adapter Sequence Read 2—[Optional] Enter the Read 2 adapter sequence of the index adapter kit.
- Barcode Mismatches Index 1—[Optional] Select from preset options (0, 1, 2). Leave it blank if you are unsure.
- Barcode Mismatches Index 2—[Optional] Select from preset options (0, 1, 2). Leave it blank if you are unsure.
- Override Cycles—[Optional] String used to specify UMI cycles and mask out cycles of a read (eg, N1Y150;I8;I7N1;Y141U10). Leave it blank if you are unsure.
- Instructions — Read only.

8. On the Record Details screen, select Validate Run Setup and Create Planned Run.
   This selection triggers the automation script, which does the following actions:
   - Validates the parameters entered on the Record Details screen.
   - Create the planned run and send it to ICA if Run Mode is Hybrid or Cloud.
   - Generates the sample sheet and attaches it to the placeholder in the Files area of the Record Details screen for all Run Mode types.
9. Select Next Steps.
   On the Assign Next Steps screen, the next step for samples is set to the next step in the workflow. The next step is AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3).
10. Select Finish Step.

At the end of this step, the pool of samples automatically advances to and is queued for the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3) step. The sequencing run is ready to be started. For details on how to start the sequencing run for different run modes, refer to *NextSeq 1000/2000 Integration v2.3.1 Configuration and User Interaction* on page 1523.

Run Mode Details

The run mode applies to all runs and determines how the run and analysis parameters are configured and how to analyze data.

- Local Mode—NextSeq 1000/2000 Integration service generates the sample sheet. Run/Analysis configuration is imported manually into NextSeq 1000/2000 instrument via a sample sheet to start the run. The downstream secondary analysis is done using the onboard DRAGEN module.

- Hybrid Mode—NextSeq 1000/2000 Integration service sends the run/analysis configuration to Illumina Connected Analytics. Run/Analysis configuration is downloaded to NextSeq 1000/2000 instrument via ICA to start the run. The downstream secondary analysis is done using the onboard DRAGEN module.
- Cloud Mode—NextSeq 1000/2000 Integration service sends the run/analysis configuration to ICA. Run/Analysis configuration is downloaded to NextSeq 1000/2000 instrument via ICA to start the run. The downstream secondary analysis is performed in the cloud.

Step 3: AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3)

This step is fully automated.

> Do not add samples to the Ice Bucket or start or complete the step. The integration completes these actions automatically.

The integration starts the step automatically and data from the run is parsed back to Clarity LIMS. No user interaction is required. However, you can open and review the various stages of the step in Clarity LIMS. Do not perform any action when reviewing the data.

Read summary metrics are recorded for the library pool in the Step Details section and the Sample Details table.

Values are populated in the following master step fields:

- Run Name
- Current Read
- Flow Cell ID
- Reagent Cartridge Lot Number
- Instrument ID
- Run Status
- Current Cycle
- Flow Cell Lot Number
- Instrument Platform
- Instrument Control Software Version
- Output Folder
- Secondary Analysis Workflow
- Reagent Cartridge ID
- Instrument Type
- RTA Version

- Sequencing Log

The summary metrics (per run level) populate in the following global fields.

- % Bases >=Q30 R1
- % Bases >=Q30 R2
- % Error Rate R1
- % Error Rate R2
- Yield (Gb) R1
- Yield (Gb) R2
- Reads PF R1
- Reads PF R2
- %PF R1
- %PF R2
- % Aligned R1
- % Aligned R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- Cluster Density R1
- Cluster Density R2

At the end of this step, the pool of samples automatically advances to (and queues for) the Demultiplexing (NextSeq 1000/2000 Sequencing v2.3) step.

Step 4: Demultiplexing (NextSeq 1000/2000 Sequencing v2.3)

This step is semi-automated.

For the demultiplexing information in the Sample Details table to display correctly, the following conditions must be met:

- The DRAGEN version must be earlier than v3.9.3.
- The samples in the run must not repeat.
- The run must be for the single lane flow cell (P1 or P2).

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> Do not add samples to the Ice Bucket or start or complete the step. The integration completes these actions automatically.

The integration starts the step automatically and demultiplexing data from the GenerateFASTQ secondary analysis is parsed back to Clarity LIMS. Review the data parsed and assign QC, depending on the criteria set, and complete the step.

In the Record Details screen, perform the following actions:

1. Review demultiplexing data.
   - Demultiplexing metrics are recorded for the library pool in the Sample Details table. Metrics include columns for # Reads, # Perfect Index Reads, # One Mismatch Index Reads, and # of >= Q30 Bases (PF).
   - The `Demultiplex_stats.csv` file generated by the DRAGEN onboard or Cloud analysis module is stored under the Files section.
2. Assign QC flags to all the individual sample. There are two ways of doing this step.
   a. Manually assign QC flags through the QC column in Sample Details table.
   b. Automatically assign QC flag by running Assign QC flags automation. This option is for scenarios where a huge number of libraries are involved.

   In the Step Details section, the following step fields are visible. (N is the number of criteria. You can use one or more criteria.)
   - Criteria N - Source Data Field—Select from preset options eg # Reads.
   - Criteria N - Operator—Select from preset options, for example, >= (greater than or equal to).
   - Criteria N - Threshold Value—Enter the desired threshold value.
3. After filling up the criteria fields, select Assign QC flags. This selection triggers the automation script, which loops through each library in the pool and apply QC flag base on the criteria set previously.
   This automation also generates an AssignQC Result file under Files section.
4. Select Next Steps.
   On the Assign Next Steps screen, the Next Step field for all samples is prepopulated with Mark protocol as complete.
5. Select Finish Step.

At this point, the whole NextSeq 1000/2000 Integration workflow is fully validated.

Troubleshooting

If an automation trigger does not appear to run its corresponding scripts, refer to *Troubleshooting Automation* in the Clarity LIMS API documentation.

If the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3) step starts but does not finish, complete the following steps:

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1. Log in to Clarity LIMS using the default user account and use one of the following methods to open the step in Clarity LIMS:
   - Method 1: In Lab View, find the step in the Recent Activities pane.
   - Method 2: Search for the step in Clarity LIMS using reagent cartridge barcode as the search term.
2. On the Record Details screen, locate the Sequencing Log field.
   The multiline text field contains logging information.

If you cannot reach the Record Details screen, or if the Sequencing Log field does not contain enough information to resolve the issue, contact the Clarity LIMS support team. Supply the relevant information from the troubleshooting steps already performed.

For integrations that run on Clarity LIMS v5.4, the events in the RECENT ACTIVITIES panel can reflect as the API access user instead of the user who ran the step. This is fixed in Clarity LIMS v6.0.

NextSeq 1000/2000 Integration v2.3.1 Release Notes

These Release Notes describe the key changes to software components for the Clarity LIMS NextSeq 1000/2000 Integration Package v2.3.1.

Compatibility

This version of the integration package is compatible with the following software:

- Clarity LIMS v5.3 and later (up to v6.1 only)
- NextSeq 1000/2000 Instrument Control Software (NCS) v1.5.0

New Features

- Bug fixes

Defect Repairs

- Fixed issue where the wrong project name is assigned to samples in the sample sheet when a run involves more than 200 samples across multiple projects.
- Fixed missing BCL convert versions in the Cloud Analysis Version custom field options in the Load to Reagent Cartridge step.

Known Issues

- Sequencing run error triggered by disk space full on instrument causes `RunParameters.xml` to be empty and the run event cannot be processed on Clarity LIMS. This error is unlikely to occur as pre-run checks on the instrument check for sufficient disk space before run is started.

Limitations

For the demultiplexing information in the Sample Details table to display correctly, the following conditions must be met:

- The DRAGEN version must be earlier than v3.9.3.

- The samples in the run must not repeat.
- The run must be for the single lane flow cell (P1 or P2).

NextSeq 1000/2000 Integration v2.3 Configuration and User Interaction

The Illumina NextSeq 1000/2000 Integration Package v2.3 supports the integration of Clarity LIMS to Illumina NextSeq 1000/2000 sequencing systems.

For instructions on validating and troubleshooting the NextSeq 1000/2000 Integration, refer to *NextSeq 1000/2000 Integration v2.3 Validation and Troubleshooting* on page 1606.

The configuration provided in this integration has been established to support NextSeq 1000/2000 lab processes. Any configuration changes to protocols or workflows—including renaming protocols, steps, and fields—could break the process.

Overview

The integration includes the following features:

- Preconfigured NextSeq 1000/2000 Sequencing v2.3 workflow that maps to lab protocols and instrument runs.
- The following preconfigured protocols:
    - Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3)
    - Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3)
    - AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3)
    - Demultiplexing (NextSeq 1000/2000 Sequencing v2.3)
- Automatic validation of run setup information before sample sheet generation or planned run creation.
- Automated generation of a sample sheet. The sample sheet is used to start the sequencing run on NextSeq 1000/2000 Control Software (NCS) via Local run mode.
- Automated creation of a planned run on Illumina Connected Analytics. The NCS retrieves and starts the planned run via Hybrid or Cloud run mode.
- Automated tracking of the NextSeq 1000/2000 sequencing run and parsing of run statistics from BaseSpace Sequence Hub into Clarity LIMS using SIS integration service.
- Automated tracking of the secondary analysis using DRAGEN (up to BCL Convert only) and parsing of demultiplexing metrics from BaseSpace Sequence Hub into Clarity LIMS using SIS integration service.
- Unicode Parameter XML file support from NCS v1.5.

- [Optional] Preconfigured Library Prep Validation v2.2.0 workflow used for validation purposes only. The workflow contains a single-step protocol that models the library prep workflow required to produce libraries tagged with index sequences. At the end of the step, these libraries are routed to NextSeq 1000/2000 Sequencing v2.3 workflow. For more information, refer to *NextSeq 1000/2000 Integration v2.3 Validation and Troubleshooting* on page 1606.
- [Optional] An integration of Clarity LIMS product analytics that make Clarity LIMS data available to the ICA and product analytics services. A subscription to ICA is required for this integration. This integration also requires installation and configuration. For more information, refer to *Clarity LIMS Product Analytics Integration v1.0 Configuration and User Interaction* on page 2425.

Information on these features is provided in the following sections.

Prerequisites and Assumptions

The Illumina NextSeq 1000/2000 Integration Package v2.3 is compatible with Illumina cloud hosted deployments only.

It is assumed that samples entering the NextSeq 1000/2000 Sequencing v2.3 workflow have gone through library preparation and quantification processes. Before they are assigned to the workflow, samples have completed the following actions:

- Samples have been accessioned into Clarity LIMS.
- Samples have been run through QC and library prep.
- Samples have the Molarity (nM) global field set to some value.
  - The Calculate Volumes automation in the Library Pooling and Dilution step requires a value in the Molarity (nM) global field.

For more information on sample accessioning, refer to *Sample Accessioning* and *Upload and Modify Samples* in the Getting Started section of the Clarity LIMS Core Functionality documentation.

You can assign samples to workflows automatically, using a routing script, or manually—from the Projects & Samples dashboard. Refer to *Assign and Process Samples* in the Clarity LIMS Core Functionality documentation.

Workflows, Protocols, and Steps

The NextSeq 1000/2000 Integration Package v2.3 includes the following workflows:

- NextSeq 1000/2000 Sequencing v2.3

  Samples are routed to this protocol after they have gone through Library Prep Validation v2.2.0 (or any other library prep workflow). Samples are pooled, diluted to final loading concentration, and added to a reagent cartridge in preparation for the sequencing run.
- [Optional] Library Prep Validation v2.2.0 - recommended for validation purposes.

The following table lists and describes the protocols and steps included in these workflows.

The workflow configuration includes a global field named Illumina Universal Sample Identifier. This text field is optional and reserved for a future feature. The field value is not required for this integration.

Table 327 Illumina NextSeq 1000/2000 Integration Package v2.3 Protocols and Steps

Protocol Name	Protocol Purpose	Steps Included in Protocol
Library Prep Validation v2.2.0 Workflow		
Library Prep Validation v2.2.0	Included for validation purposes only, this protocol models the library prep steps required to advance samples to the NextSeq 1000/2000 Sequencing v2.3 workflow. The protocol contains a single step—Library Prep Validation v2.2.0. At the end of this step, a routing script sends the samples to the first step of the NextSeq 1000/2000 Sequencing v2.3 workflow. The first step is Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3).	1. Library Prep Validation v2.2.0
NextSeq 1000/2000 Sequencing v2.3 Workflow		

Protocol Name	Protocol Purpose	Steps Included in Protocol
NextSeq 1000/2000 Sequencing v2.3	This protocol models the lab processes of starting a NextSeq 1000/2000 sequencing run. Details of the steps: 1. Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3) a. Samples are pooled and diluted to the final loading concentration with the help of the Calculate Volume script. 2. Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3) a. The library pool from step 1 is then ready to be loaded to the NextSeq 1000/2000 reagent cartridge. b. Run and analysis information is validated. c. Sample sheet is generated and/or planned run is created. 3. AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3) a. This step is a fully automated step that is started and completed automatically after the sequencing run is started and completed on the instrument side. b. All the metadata (eg, run configuration, primary run metrics, etc.) of the sequencing run are recorded automatically. Do not add samples to the Ice Bucket or start or complete the step. The integration completes these actions automatically. c. If GenerateFastQ secondary analysis is not planned for the run, samples complete the	1. Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3) 2. Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3) 3. AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3) 4. Demultiplexing (NextSeq 1000/2000 Sequencing v2.3)

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Validation Workflow

The Library Prep Validation v2.2.0 workflow allows for validation of the system after installation is complete. For details, refer to *NextSeq 1000/2000 Integration v2.3 Validation and Troubleshooting* on page 1606*NextSeq 1000/2000 Integration v2.2 Validation and Troubleshooting* on page 1651

Step Automations

Automation details are provided in the sections following the table.

For details on the validation workflow, refer to *NextSeq 1000/2000 Integration v2.3 Validation and Troubleshooting* on page 1606.

Importing either the Library Prep Validation v2.2.0 workflow or the NextSeq 1000/2000 Sequencing v2.3 workflows provides a Global field named Illumina Universal Sample Identifier. This is a text field that is reserved for Clarity LIMS product analytics support and is optional. The value is not required for this integration.

Table 328 NextSeq 1000/2000 Sequencing v2.3 Workflow Step Automations

Protocol	Step	Automation
NextSeq 1000/2000 Sequencing v2.3	Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3)	• Calculate Volumes • Register Pools & Register Step Completed[1] • Register Step Started[1] • Set Next Step
	Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3)	• Register Step Completed[1] • Retrieve Analysis Workflow Versions • Set Next Step • Validate Reagent Cartridge Barcode • Validate Run Setup and Create Planned Run • Validate Single Input & Register Step Started[2]
	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3)	• Register NextSeq1k2k RunCompleted & Register Step Completed[1] • Register Step Started & Register NextSeq1k2k Run & Register NextSeq1k2k Run Association & Register NextSeq1k2k RunStarted[1]
	Demultiplexing (NextSeq 1000/2000 Sequencing v2.3)	• Assign Demultiplex QC flags • Register Analysis Completed & Register Step Completed[1] • Register Step Started[1] • Set Next Step

[1] These automations are required for Clarity LIMS product analytics support only.

[2] These automations are required for the NextSeq 1000/2000 Sequencing v2.3 workflows and contain additional logic needed for Clarity LIMS product analytics support. If you would like to remove Clarity LIMS product analytics support, contact Illumina Support.

Terminology

The Illumina NextSeq 1000/2000 Integration Package v2.3 supports Clarity LIMS v5.3 and later.

As of Clarity LIMS v5, the term user-defined field (UDF) has been replaced with custom field in the user interface. However, the API resource is still called UDF.

There are two types of custom fields:

- Master step fields—Configured on master steps. Master step fields only apply to the following:
  - The master step on which the fields are configured.
  - The steps derived from those master steps.
- Global fields—Configured on entities (eg, submitted sample, derived sample, measurement, etc.). Global fields apply to the entire Clarity LIMS system.

Step 1: Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3)

In this step, the addition of RSB dilutes pooled samples. Manually create a working pool based on the final loading concentration required.

Only create one pool per step for the Calculate Volume automation script to work properly.

Register Step Started

This automation registers the start time of the step by publishing messages to UPA through the LINK service. This automation is automatically triggered on the step. It is only used for UPA support.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_started.groovy'
```

Calculate Volumes Automation

Select the Calculate Volumes button on the Record Details screen to trigger this automation automatically, which does the following actions:

- Sets RSB Volume for Pool (µl) field value to 24 for calculation purpose of the 2 nM intermediate library pool.
- Copies the Final Loading Concentration (pM) from step inputs to step outputs.

```
-exp 'step.::RSB Volume for Pool (µl):: = 24; output.::Final Loading
Concentration (pM):: = step.::Final Loading Concentration (pM)::'
```

- Calculate per sample volume required for each library to make the 2 nM intermediate library pool (24ul in volume).

```
-exp 'input.::Per Sample Volume (µl):: = ( 48/ input.::Molarity (nM):: );
step.::RSB Volume for Pool (µl):: -= input.::Per Sample Volume (µl)::'
```

- Calculate the volume required of the 2 nM library pool to be diluted further to the Final Loading Concentration (pM) with the Final Loading Volume (µl).

```
-exp 'step.::Library Pool Volume (µl):: = (step.::Final Loading Concentration
(pM):: * step.::Final Loading Volume (µl)::/2000); output.::RSB Volume (µl):: =
step.::Final Loading Volume (µl):: - step.::Library Pool Volume (µl)::'
```

- Uses the `NextSeq1K2K_Pool1.csv`, `NextSeq1K2K_Pool2.csv`, and `NextSeq1K2K_Pool3.csv` template files to generate a single CSV file containing information about the pool and the samples it contains. The generated file is stored in the Calculation File placeholder, in the Files section, for download.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} \
   script:driver_file_generator \
      -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NextSeq1K2K_
      Pool1.csv \
      -o 1.csv \
   script:driver_file_generator \
      -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NextSeq1K2K_
      Pool2.csv \
      -o 2.csv \
   script:driver_file_generator \
      -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NextSeq1K2K_
      Pool3.csv \
      -o 3.csv \
&& cat 1.csv 2.csv 3.csv > {compoundOutputFileLuid0}.csv
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol. The next step is Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3):

```
nextStep = ::ADVANCE::
```

Register Pools & Register Step Completed

- This automation is automatically triggered on exit of the step and performs the following tasks:

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- Registers the pool and library information used by the Clarity LIMS workflow. Samples in the pool are assumed to have gone through library preparation.
- Registers the completion time of the step by publishing messages to UPA through the LINK service.

This automation is only used for UPA support.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/pooling.groovy' && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_completed.groovy'
```

Master Step Fields

There are four fields defined on the Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3) step.

Table 329 Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3) Master Step Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Final Loading Concentration (pM)	Numeric Dropdown	Required: Yes Read Only: No Custom Entries: Yes	Presets • 650 • 750 • 1000 • 2000
Final Loading Volume (µl)	Numeric	Required: Yes Read Only: No	Default • 24

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Library Pool Volume (µl)	Numeric	Required: No Read Only: Yes	Hidden ⚠ This field is used by the Calculate Volumes automation. Removing, renaming, or modifying the field will break the automation.
RSB Volume for Pool (µl)	Numeric	Required: No Read Only: Yes	Hidden ⚠ This field is used by the Calculate Volumes automation. Removing, renaming, or modifying the field will break the automation.

Global Fields

The following table lists the global fields that are configured to display on the Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3) step.

Table 330 Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3) Global Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Final Loading Concentration (pM)	Numeric Dropdown	Required: Yes Read Only: No Custom Entries: Yes	Decimal Places Displayed: 0 Presets • 225 • 400
RSB Volume (µl)	Numeric	Required: No Read Only: Yes	Decimal Places Displayed: 2

User Interaction

Refer to *NextSeq 1000/2000 Integration v2.3 Validation and Troubleshooting* on page 1606 for details.

Step 2: Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3)

In this step, scan the reagent cartridge barcode into Clarity LIMS, then manually place the working pool into the reagent cartridge for the NextSeq 1000/2000 run. This step validates the run setup and analysis information and generates the sample sheet file and/or creates a planned run on Illumina Connected Analytics, depending on the Run Mode selected.

The NextSeq 1000/2000 reagent cartridges support different number of read cycles and these values are indicated on the box pill, bag pill, and cartridge pill labels. Make sure that the read values configured for the planned run are within the maximum allowable reads for the cartridge type.

Validate Single Input Automation & Register Step Started

Automatically triggered at the beginning of the step, this automation does the following actions:

- Checks that there is only one container input to the step. This script is required for the NextSeq 1000/2000 Sequencing v2.3 workflow.

```
script:validateSampleCount -min 1 -max 1
```

- Registers the start time of the step by publishing messages to UPA through the LINK service. This script is only used for Clarity LIMS product analytics support.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_
started.groovy'
```

Validate Reagent Cartridge Barcode Automation

Automatically triggered on exit of the Placement screen, the following automation validates the reagent cartridge barcode to make sure it conforms to the barcode mask [A-Za-z]{2}[0-9]{7}-[A-Za-z0-9]{4}:

```
if (!output.container.name.matches( ::[A-Za-z]{2}[0-9]{7}-[A-Za-z0-9]{4}:: ) )
{fail ( ::Invalid Reagent Cartridge Barcode. Please verify and try again.:: ) }
```

Retrieve Analysis Workflow Versions Automation

Automatically triggered upon entry of the Record Details screen, this automation:

Fetches the available analysis workflow versions from ICA and updates the preset values of both Local Analysis Workflow Versions & Cloud Analysis Workflow Versions fields.

```
/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/nextseq1k2k-
automation.jar script:retrieve_analysis_workflow_versions -i {stepURI:v2} -u
{username} -p {password} -l {compoundOutputFileLuid2}
```

Do not modify or disable this automation script as it breaks the integration.

Validate Run Setup and Create Planned Run Automation

Automatically triggered when a button on the Record Details screen is selected, this automation does the following actions:

- Validates the parameters entered on the Record Details screen. These parameters are used to set up the run and generate the sample sheet file and/or create the planned run on ICA.
  - Run Name can only contain alphanumeric, dash, underscore, or period characters. Spaces are not permitted.

```
if (!step.::Run Name::.matches(::[a-zA-Z0-9-_.]+::))
{ fail(::Run Name contains prohibited characters. Allowed characters are: a-z,
A-Z, 0-9, -, _, and .::) }
```

  - Run Name must not exceed 255 characters.

```
if (step.::Run Name::.length() > 255) { fail(::Run Name shall not exceed 255
characters.::) };
```

- Checks the Index Reads, Index Read 1, and Index Read 2 field values.

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  - If Index Reads is No Index, Index Read 1 and Index Read 2 values must be 0 (error results if it is otherwise).
  - If Index Reads is Single Index, Index Read 1 value must be greater than 0, and Index Read 2 values must be 0 (error results if it is otherwise).
  - If Index Reads is Dual Index, Index Read 1 and Index Read 2 values must be greater than 0 (error results if it is otherwise).

```
if (step.::Index Reads:: == ::No Index::)
{ if (step.::Index Read 1:: != 0 || step.::Index Read 2:: != 0) { fail(::Index Read 1 and Index Read 2 must be 0 if the Index Reads is No Index.::) } }
else
{ if (step.::Index Reads:: == ::Single Index::)
{ if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) { fail(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if the Index Reads is Single Index.::) } }
else
{ if (step.::Index Read 1:: == 0 || step.::Index Read 2:: == 0) { fail(::Index Read 1 and Index Read 2 must be greater than 0 if the Index Reads is Dual Index.::) } } }
```

- Checks the Paired End, Read 2 Cycles, and Index Read 2 field values.
  - If Paired End is set to True and Read 2 Cycles value is 0, an error is generated.
  - If Paired End is set to False and Read 2 Cycles or Index Read 2 values are greater than 0, an error is generated.

```
if (step.::Paired End::.toBoolean())
{ if (step.::Read 2 Cycles:: == 0) { fail(::Read 2 Cycles must not be zero if it is Paired End read.::) }}
else
{if (step.::Read 2 Cycles:: != 0 || step.::Index Read 2:: != 0) {fail(::Read 2 Cycles and Index 2 Cycles must be 0 if it is not Paired End Read.:: )}}
```

- Checks the Adapter Sequence Read 1 and Adapter Sequence Read 2 field values.
  - Adapter Sequence Read 1 and Adapter Sequence Read 2 can only contain ACTG+ characters.

```
if (step.hasValue(::Adapter Sequence Read 1::) && !step.::Adapter Sequence Read 1::.matches(::[ACTG+]+::)) { fail(::Adapter Sequence Read 1 contains prohibited characters. Allowed characters are: ACTG+::) };
if (step.hasValue(::Adapter Sequence Read 2::) && !step.::Adapter Sequence Read 2::.matches(::[ACTG+]+::)) { fail(::Adapter Sequence Read 2 contains prohibited characters. Allowed characters are: ACTG+::) }
```

- Checks Override Cycles field value.
  - Override Cycles can only contain Y, N, I, U, 0–9, and semicolon characters.

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```
if (step.hasValue(::Override Cycles::) && !step.::Override Cycles::.matches(::
[YNIU0-9;]+::))
{ fail(::Override Cycles contains prohibited characters. Allowed characters are:
Y, N, I, U, 0-9 and ;. Example: N1Y150;I8;I7N1;Y141U10.::) }
```

- When the Run Mode is Cloud, the script does the following actions:
  - Sets Local Analysis Workflow Versions to None.
  - If the Cloud Analysis Workflow Versions is None, the script displays the following error: `Invalid option selected for Cloud Analysis Workflow Versions field.`

```
if (step.::Run Mode:: == ::Cloud::) { step.::Local Analysis Workflow Versions::
= ::None::; if (step.::Cloud Analysis Workflow Versions:: == ::None::) { fail
(::Invalid option selected for Cloud Analysis Workflow Versions field.::); }};
```

  - Due to automation script limitation, a validation rule resides in the backend service to perform the following action: If Cloud Analysis Workflow Versions is 3.7.4 or later, set FASTQ Compression Format to gzip.
- When the Run Mode is Local or Hybrid, the script does the following actions:
  - Sets Cloud Analysis Workflow Versions to None.
  - If Local Analysis Workflow Versions is None, the script displays the following error message: `Invalid option selected for Local Analysis Workflow Versions field.`

```
if (step.::Run Mode:: == ::Local:: || step.::Run Mode:: == ::Hybrid::) {
step.::Cloud Analysis Workflow Versions:: = ::None::; if (step.::Local Analysis
Workflow Versions:: == ::None::) { fail(::Invalid option selected for Local
Analysis Workflow Versions field.::); }};
```

  - Due to automation script limitation, a validation rule resides in the backend service. If Local Analysis Workflow Versions is 3.7.4 or later, an error occurs if FASTQ Compression Format is None.
- Generates the sample sheet and/or creates the planned run on ICA.
  - Sample sheet is attached to the step.

```
/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/nextseq1k2k-
automation.jar
script:generate_v2_sample_sheet -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid2} -c {compoundOutputFileLuid0}
```

  - There is backend validation of the sample sheet content. This validation makes sure that the sample sheet is valid to set up a Local Mode run or is ready for DRAGEN applications. Refer to *NextSeq 1000/2000 Integration v2.3 Validation and Troubleshooting* on page 1606 for submitted sample name character restrictions.

Set Next Step Automation

Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol—AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3).

```
nextStep = ::ADVANCE::
```

Register Step Completed

Automatically triggered on exit of the step, this automation registers the completion time of the step by publishing messages to Clarity LIMS product analytics through the LINK service. This script is only used for Clarity LIMS product analytics support.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_completed.groovy'
```

Master Step Fields

There are 24 fields defined on the Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3) step. These fields are required for sample sheet generation and planned run creation on ICA.

Table 331 Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3) Master Step Fields Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Run Name	Text	Required: Yes Read Only: No	
Instrument Type	Text Dropdown	Required: Yes Read Only: No Custom Entries: No	Presets • NextSeq1000 • NextSeq2000
Run Mode	Text Dropdown	Required: Yes Read Only: No Custom Entries: No	Presets • Local • Hybrid • Cloud
Paired End	Text Dropdown	Required: Yes Read Only: No Custom Entries: No	Presets • True • False

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Read 1 Cycles	Numeric Dropdown	Required: Yes Read Only: No Custom Entries: Yes	Presets • 301 • 151 • 101 • 51
Read 2 Cycles	Numeric Dropdown	Required: Yes Read Only: No Custom Entries: Yes	Presets • 301 • 151 • 101 • 51
Index Reads	Text Dropdown	Required: Yes Read Only: No Custom Entries: No	Presets • No Index • Single Index • Dual Index
Index Read 1	Numeric Dropdown	Required: Yes Read Only: No Custom Entries: Yes	Presets • 0 • 6 • 8
Index Read 2	Numeric Dropdown	Required: Yes Read Only: No Custom Entries: Yes	Presets • 0 • 6 • 8
Analysis Workflow	Text	Required: Yes Read Only: Yes	Default • GenerateFASTQ
Adapter Sequence Read 1	Text	Required: No Read Only: No	
Adapter Sequence Read 2	Text	Required: No Read Only: No	
Barcode Mismatches Index 1	Numeric Dropdown	Required: No Read Only: No Custom Entries: No	Presets • 0 • 1 • 2

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Barcode Mismatches Index 2	Numeric Dropdown	Required: No Read Only: No Custom Entries: No	Presets • 0 • 1 • 2
Override Cycles	Text	Required: No Read Only: No	
Cloud Run ID	Text	Required: No Read Only: Yes	Hidden - Used in creating planned run in ICA. Do not delete, rename, or modify. These actions will break the integration.
Use Custom Index Read 1 Primer	Toggle Switch	Required: No Read Only: No	Hidden Custom primer option is currently not supported. Do not enable as it will break the integration.
Use Custom Index Read 2 Primer	Toggle Switch	Required: No Read Only: No	Hidden Custom primer option is currently not supported. Do not enable as it will break the integration.

For Research Use Only. Not for use in diagnostic procedures.

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Use Custom Read 1 Primer	Toggle Switch	Required: No Read Only: No	Hidden Custom primer option is currently not supported. Do not enable as it will break the integration.
Use Custom Read 2 Primer	Toggle Switch	Required: No Read Only: No	Hidden Custom primer option is currently not supported. Do not enable as it will break the integration.
Instructions	Multi-Line Text	Required: No Read Only: Yes	Default • If GenerateFASTQ analysis is required, select the DRAGEN version in Cloud Analysis Workflow Versions (for Cloud run mode) or Local Analysis Workflow Versions (for Local/Hybrid run mode) custom fields.
Local Analysis Workflow Versions	Text Dropdown	Required: No Read Only: No Custom Entries: No	Default • None

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Cloud Analysis Workflow Versions	Text Dropdown	Required: No Read Only: No Custom Entries: No	Default • None
FASTQ Compression Format	Text Dropdown	Required: Yes Read Only: No Custom Entries: No	Default • gzip (default value) • DRAGEN • None

Do not modify the configuration of the Local Analysis Workflow Versions and Cloud Analysis Workflow Versions fields. Modifying these fields can break the integration.

User Interaction

Refer to *NextSeq 1000/2000 Integration v2.3 Validation and Troubleshooting* on page 1606 for details.

Step 3: AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3)

This step is fully automated.

The integrations start and complete the step automatically and data from the run is parsed back to Clarity LIMS. No user interaction is required. In this step, the pooled samples in the reagent cartridge are sequenced on the NextSeq 1000/2000 instrument.

Do not add samples to the Ice Bucket or start or complete the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3) step. The integration does this step automatically.

Register Step Started & Register NextSeq1k2k Run & Register NextSeq1k2k Run Association & Register NextSeq1k2k RunStarted

Automatically triggered at the beginning of the step, this automation performs the following actions:

- Registers the start time of the step.
- Registers the NextSeq 1000/2000 sequencing run configuration.
- Registers the association of the sequencing run with the actual samples by linking the Instrument Run ID with the IDs for the samples.
- Registers the time that the sequencing run starts.

These actions are done by publishing messages to Clarity LIMS product analytics through the LINK service. This automation is only used for Clarity LIMS product analytics support.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/common/step_
started.groovy' && /opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/unified-product-analytics/automation/unified-
product-analytics-automation.jar script:executeUPAAutomationScript -i
{stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0} -s
'com/illumina/upa/scripts/nextseq1k2k/register_nextseq1k2k_run.groovy' &&
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/nextseq1k2k/nextseq1k2k_
associate_seqrun.groovy' && /opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/unified-product-analytics/automation/unified-
product-analytics-automation.jar script:executeUPAAutomationScript -i
{stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0} -s
'com/illumina/upa/scripts/nextseq1k2k/nextseq1k2k_seqrun_started.groovy'
```

Register NextSeq1k2k RunCompleted & Register Step Completed

- Automatically triggered upon exiting the step, this automation performs the following actions:
- Registers the sequencing run completion time, run status, and run metrics. This script requires the Cloud Run ID field from the Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3) step.
- Registers the completion time of the step.

These actions are done by publishing messages to Clarity LIMS product analytics through the LINK service. This automation is only used for Clarity LIMS product analytics support.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/nextseq1k2k/nextseq1k2k_
seqrun_completed.groovy' && /opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/unified-product-analytics/automation/unified-
product-analytics-automation.jar script:executeUPAAutomationScript -i
{stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0} -s
'com/illumina/upa/scripts/common/step_completed.groovy'
```

Master Step Fields

There are 24 fields defined on the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3) step. These fields are used to display the run status and sequencing run and analysis configuration parsed from the `RunParameters.xml` file of the sequencing run.

Do not modify the name of any of the master step fields. This action causes the integration to break.

Run Parameters and Corresponding Clarity LIMS Step Fields

The following table shows how some of the step fields map to the fields on the `RunParameters.xml` file, and whether the field is visible on the Record Details screen.

Master Step Field	RunParameters.xml Field	On Record Details Screen
Current Cycle	Calculated based on CompletedCycles Field	Visible
Current Read	Calculated based on CompletedCycles field against PlannedCycles	Visible
Flow Cell ID	FlowCellSerialNumber	Visible
Flow Cell Lot Number	FlowCellLotNumber	Visible
Instrument Control Software Version	ApplicationVersion	Visible
Instrument ID	InstrumentSerialNumber	Visible
Output Folder	OutputFolder	Visible
Reagent Cartridge ID	CartridgeSerialNumber	Visible
Reagent Cartridge Lot Number	CartridgeLotNumber	Visible
RTA Version	RtaVersion	Visible
Run Name	ExperimentName	Visible
Secondary Analysis Workflow	SecondaryAnalysisWorkflow	Visible

Master Step Field	RunParameters.xml Field	On Record Details Screen
Flow Cell Mode	FlowCellMode	Hidden Do not delete, rename, or modify. These actions can break the integration.
Instrument Run ID	Derived from OutputFolder	Hidden Do not delete, rename, or modify. These actions can break the integration.
Run End Time	RunEndTime	Hidden Do not delete, rename, or modify. These actions can break the integration.
Run Start Time	RunStartTime	Hidden Do not delete, rename, or modify. These actions can break the integration.
Secondary Analysis Mode	SecondaryAnalysisMode	Hidden Do not delete, rename, or modify. These actions can break the integration.
Secondary Analysis Platform Version	SecondaryAnalysisPlatformVersion	Hidden Do not delete, rename, or modify. These actions can break the integration.

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Master Step Field	RunParameters.xml Field	On Record Details Screen
SkipObdd	SkipObdd	Hidden Do not delete, rename, or modify. These actions can break the integration.

Additional Master Step Fields and Values

The following table shows how the other step fields derive their values, and whether the step field is visible on the Record Details screen.

Master Step Field	RunParameters.xml Field	On Record Details Screen
Instrument Platform	NextSeq 1000/2000 Constant value	Visible
Instrument Type	One of the following options: • NextSeq1000 • NextSeq2000 Determined from Instrument Type step field from previous Load to Reagent Cartridge step.	Visible
Run Status	One of the following options: • RunStarted • RunCompletedSuccessfully • RunAbortedByUser • RunErroredOut Set by the SIS integration service*	Visible
Sequencing Log	Filled in by the SIS integration service* as the sequencing run proceeds	Visible
BaseSpace Run ID	Received from SQS message in the SIS integration service	Hidden Do not delete, rename, or modify. These actions will break the integration.

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*For information on how the integration works, refer to *Run Status, Primary Metrics and Analysis Results Parsing and Recording* on page 1642.

Global Fields

The following global fields are used to capture the run metrics in Clarity LIMS.

- % Bases >=Q30 R1
- % Bases >=Q30 R2
- % Error Rate R1
- % Error Rate R2
- Yield (Gb) R1
- Yield (Gb) R2
- Reads PF R1
- Reads PF R2
- %PF R1
- %PF R2
- % Aligned R1
- % Aligned R2
- % Phasing R1
- % Phasing R2
- % PrePhasing R1
- % PrePhasing R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- Cluster Density R1
- Cluster Density R2

At the end of this step, the pool of samples is automatically advanced to (and queued for) the Demultiplexing (NextSeq 1000/2000 Sequencing v2.3) step.

Step 4: Demultiplexing (NextSeq 1000/2000 Sequencing v2.3)

This step is a semi-automated step.

The integration starts the step automatically and demultiplexing data from the GenerateFASTQ secondary analysis is parsed back to Clarity LIMS. The lab scientist reviews the demultiplexing result parsed into Clarity LIMS, assigns QC flags, and completes the step.

For the demultiplexing information in the Sample Details table to display correctly, the following conditions must be met:

- The DRAGEN version must be earlier than v3.9.3.
- The samples in the run must not repeat.
- The run must be for the single lane flow cell (P1 or P2).

Do not add samples to the Ice Bucket or start or complete the step. The integration completes these actions automatically.

Register Step Started Automation

Automatically triggered at the beginning of the step, this automation registers the start time of the step by publishing messages to UPA through the LINK service. This automation is only used for UPA support.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_started.groovy'
```

Assign Demultiplexing QC Flags Automation

Automatically triggered when you select a button on the Record Details screen, this automation assigns QC flags based on the criteria set in the step fields.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -u {username} -p {password} script:assignQCForDemuxStep -i {processURI:v2} -log {compoundOutputFileLuid1} -qcResult {compoundOutputFileLuid2}
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation sets the next step to Advance and the samples to complete the protocol.

```
nextStep = ::ADVANCE::
```

Register Analysis Completed & Register Step Completed Automation

Automatically triggered on exit of the step, this automation performs the following actions:

- Registers the NextSeq 1000/2000 analysis status and result.
- Registers the completion time of the step.

These actions are done by publishing messages to UPA through the LINK service. This automation is only used for UPA support.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_
completed.groovy' && /opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/unified-product-analytics/automation/unified-
product-analytics-automation.jar script:executeUPAAutomationScript -i
{stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s
'com/illumina/upa/scripts/nextseq1k2k/nextseq1k2k_analysisrun_completed.groovy'
```

Master Step Fields

The following table lists the 12 master step fields that are configured on the Demultiplexing (NextSeq 1000/2000 Sequencing v2.3) master step.

Table 332 Master Step Field Configuration for Demultiplexing (NextSeq 1000/2000 Sequencing v2.3) Step

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Criteria 1 - Source Data Field	Text Dropdown	Required: No Read Only: No Custom Entries: Yes	Presets • # Reads • # Perfect Index Reads • # One Mismatch Index Reads • # of >= Q30 Bases (PF)
Criteria 2 - Source Data Field	Text Dropdown	Required: No Read Only: No Custom Entries: Yes	Presets • # Reads • # Perfect Index Reads • # One Mismatch Index Reads • # of >= Q30 Bases (PF)

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Criteria 1 - Operator	Text Dropdown	Required: No Read Only: No Custom Entries: Yes	Presets • >= • <= • = • !=
Criteria 2 - Operator	Text Dropdown	Required: No Read Only: No Custom Entries: Yes	Presets • >= • <= • = • !=
Criteria 1 - Threshold Value	Numeric	Required: No Read Only: No	• Valid integer value
Criteria 2 - Threshold Value	Numeric	Required: No Read Only: No	• Valid integer value
Log	Multiline Text	Required: No Read Only: Yes	
Analysis Completed Time	Text	Required: No Read Only: Yes	Hidden Do not delete, rename, or modify. These actions will break the integration.
Analysis Started Time	Text	Required: No Read Only: Yes	Hidden Do not delete, rename, or modify. These actions will break the integration.

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Analysis Status	Text	Required: No Read Only: Yes	Hidden Do not delete, rename, or modify. These actions will break the integration.
BSSH Analysis ID	Text	Required: No Read Only: Yes	Hidden Do not delete, rename, or modify. These actions will break the integration.
Reagent Cartridge ID	Text	Required: No Read Only: Yes	Hidden Used for parsing demultiplexing results Do not delete, rename, or modify. These actions will break the integration.

Global Fields

There are five fields defined on the Demultiplexing (NextSeq 1000/2000 Sequencing v2.3) step. The fields are used to display the demultiplexing result metrics for individual library in the sample pool.

The following table lists the global fields that are configured on the Demultiplexing (NextSeq 1000/2000 Sequencing v2.3) step.

Table 333 Global Field Configuration for Demultiplexing (NextSeq 1000/2000 Sequencing v2.3) Step

Field Name	Field Type	Field Constraints/Options
# of >= Q30 Bases (PF)	Numeric	Required: No Read Only: Yes
# One Mismatch Index Reads	Numeric	Required: No Read Only: Yes
# Perfect Index Reads	Numeric	Required: No Read Only: Yes
# Reads	Numeric	Required: No Read Only: Yes
Mean Quality Score (PF)	Numeric	Required: No Read Only: Yes

User Interaction

Refer to *NextSeq 1000/2000 Integration v2.3 Validation and Troubleshooting* on page 1606 for details.

How the NextSeq 1000/2000 Integration Works

Sample Sheet Generation and Planned Run Creation

On the Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3) step, when the Validate Run Setup and Create Planned Run automation is triggered, all the run and analysis parameters entered in the Run Details screen is sent to Illumina Connected Analytics. ICA validates the run and analysis configuration, and sends an error message to Clarity LIMS if the validation fails. If the validation passes, ICA generates the sample sheet (and sends back to Clarity LIMS) and/or creates a planned run depending on the Run Mode selected.

- Local run mode: Only the sample sheet is generated and stored on Clarity LIMS. The sample sheet contains all the run and analysis configuration required to start the run on the instrument.
- Hybrid/Cloud run mode: A planned run is created on ICA. This planned run contains all the run and analysis configuration required to start the run on the instrument. The sample sheet is also generated and stored on Clarity LIMS for reference purpose.

Refer to *Step-by-Step Instruction to Start A Sequencing Run On Instrument With Different Run Modes* on page 1648 for details on how to start a run using different run mode.

Run Status, Primary Metrics and Analysis Results Parsing and Recording

After the sequencing run is started on the instrument, NextSeq 1000/2000 Control Software (NCS) notifies BaseSpace Sequence Hub that the sequencing run has started.

BSSH then does the following actions:

- Local run mode: Creates a run on the BaseSpace Sequence Hub database with the run configuration information received from NCS.
- Hybrid/Cloud run mode: Creates a run on the BaseSpace Sequence Hub database by copying the run configuration information from the planned run created in ICA, using a special Cloud Run ID received from NextSeq 1000/2000 Control Software.

After the run is created on BaseSpace Sequence Hub, a run update event message that carries the RunStarted equivalent message is created on BSSH and delivered through ICA and, eventually, to Clarity LIMS SQS queue service. The SIS integration service grabs and processes the message.

Based on the reagent cartridge barcode information in the event message (refer to the following example), SIS looks in the queue of AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3) step for a container with the same reagent cartridge barcode. When identified, the step is started automatically and the Run Status field on Record Details screen is updated to RunStarted. SIS then updates all the other step fields (eg, Current Read, Output Folder) by parsing the details from the `RunParameters.xml` uploaded to BaseSpace Sequence Hub by NCS.

```
#Example of an run update event
{
"acl": [
"wid:61dc14e8-d823-3bd1-bea3-66f9c8388254"
],
"apiUrl": "https://api.basespace.illumina.com/v2/runs/r.ZvTfDXsNhUyh2kA5S4Zewg",
"dateModified": "2020-03-26T09:19:58.5546497Z",
"flowcellBarcode": "11M11COMC",
"gdsFolderPath": "/Runs/200326_MP2-15_87_11M11COMC_r.ZvTfDXsNhUyh2kA5S4Zewg",
"gdsVolumeName": "bssh.61dc14e8d8233bd1bea366f9c8388254",
"id": "r.ZvTfDXsNhUyh2kA5S4Zewg",
"instrumentRunId": "200326_MP2-15_87_11M11COMC",
"name": "200326_MP2-15_87_11M11COMC",
"reagentBarcode": "EC0001990-EC5",
"sampleSheetName": "SampleSheet.csv",
"status": "Running",
"v1pre3Id": "194424236"
}
```

How the SIS Integration Service Handles Run Status

The NCS continues to notify BSSH of any run status change throughout the course of the sequencing run. The run update event delivery process continues as described in the previous section.

The following table lists the run status details and how the SIS integration service handles each status.

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Run Status	Shown in Step	Details
RunStarted	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3)	Run Status field is updated to RunStarted. All other step fields (except Run End Time) are updated based on `RunParameters.xml` detail obtained from BaseSpace Sequence Hub, the SQS event message, and the step field value from the Load to Reagent Cartridge (NextSeq 1000/2000 v2.3) step. Reagent cartridge lot information received from BaseSpace Sequence Hub is compared against the details of the pre-accessioned reagent cartridge on Clarity LIMS. • If the expiration date is different, it is updated to the date received from BaseSpace Sequence Hub. • If reagent cartridge is not found, it is created and set to Active. • If reagent cartridge is not in Active status, it is set to Active. • Multiline Sequencing Log field is updated.

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Run Status	Shown in Step	Details
RunCompletedSuccessfully	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3)	Run Status field is updated to RunCompletedSuccessfully Current Read and Current Cycle fields are updated to the final cycle number and read number based on `RunParameters.xml` detail obtained from BaseSpace Sequence Hub. Run End Time field is updated based on RunParameters.xml detail obtained from BaseSpace Sequence Hub. Sequencing run result (primary metrics) is downloaded from BaseSpace Sequence Hub and the fields in Sample Details table (eg, % Error Rate R1, % Phasing, etc.) are updated with the values. Multiline Sequencing Log field is updated. SIS autocompletes the step and routes the samples in the reagent cartridge container to the next step (eg, Demultiplexing (NextSeq 1000/2000 Sequencing v2.3)) step.

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Run Status	Shown in Step	Details
RunErroredOut	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3)	Run Status field is updated to RunErroredOut. Current Read and Current Cycle fields are updated to the final cycle number and read number that the run stopped based on `RunParameters.xml` detail obtained from BaseSpace Sequence Hub. Run End Time field is updated based on `RunParameters.xml` detail obtained from BaseSpace Sequence Hub. Sequencing run result (primary metrics) is downloaded from BaseSpace Sequence Hub and the fields in Sample Details table (eg, % Error Rate R1, % Phasing, etc.) are updated with the values. Multiline Sequencing Log field is updated. SIS does not autocomplete the step. Complete the step manually.

Run Status	Shown in Step	Details
RunAbortedByUser	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3)	Run Status field is updated to RunAbortedByUser. Current Read and Current Cycle fields are updated to the final cycle number and read number that was aborted based on `RunParameters.xml` details obtained from BaseSpace Sequence Hub. Run End Time field is updated based on RunParameters.xml detail obtained from BaseSpace Sequence Hub. Sequencing run result (primary metrics) is downloaded from BaseSpace Sequence Hub and the fields in Sample Details table (eg, % Error Rate R1, % Phasing, etc.) are updated with the values. Multiline Sequencing Log field is updated. SIS does not autocomplete the step. Complete the step manually.
AnalysisStarted	Demultiplexing (NextSeq 1000/2000 Sequencing v2.3)	SIS starts the step automatically for the reagent cartridge container. Multiline Sequencing Log field is updated.

Run Status	Shown in Step	Details
AnalysisCompleted	Demultiplexing (NextSeq 1000/2000 Sequencing v2.3)	SIS downloads the `Demultiplex_stats.csv` file, which contains the demultiplexing result from BaseSpace Sequence Hub. The file is parsed and details are recorded in the Sample Details table for each library in the library pool. Hidden master step fields are updated with information from BaseSpace Sequence Hub. In particular, Analysis Status is updated to Complete. Multiline Sequencing Log field is updated. SIS does not autocomplete the step. Assign the QC flag to the individual library and manually complete the step.
AnalysisFailed	Demultiplexing (NextSeq 1000/2000 Sequencing v2.3)	Hidden step fields are updated with information from BaseSpace Sequence Hub. In particular, Analysis Status is updated to either TimedOut or Aborted. Multiline Sequencing Log field is updated. SIS does not auto-complete the step. Assign the QC flag to the individual library and manually complete the step.

Step-by-Step Instruction to Start A Sequencing Run On Instrument With Different Run Modes

Local Run Mode

1. On the NextSeq 1000/2000 Control Software (NCS) interface, select the menu button and select Settings.
2. Select Local Run Setup and Proactive, Run Monitoring and Storage*.
3. [Optional] Enter the default output folder location.
4. Select Save.
5. On the main screen, select Start.

6. Sign in to the BaseSpace Sequence Hub (BSSH) account that was used during NextSeq 1000/2000 Integration package installation setup.
   a. Select the workgroup (if applicable).
   b. Select Next.
7. Select Choose, and a file browser pops up.
8. Browse and select the sample sheet generated during the Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3) step.
   When selected, the sample sheet has its content validated. After the validation passes, run and analysis configuration is parsed and autopopulated in the next page.
9. Select Review to review the sequencing run settings.
10. Select Load and manually load the reagent cartridge and flow cell.
11. Select Sequence and the pre-run check proceeds.
    After the pre-run check passes, the sequencing run starts automatically.

Hybrid or Cloud Run Mode

1. On the NextSeq 1000/2000 Control Software interface, select the menu button and select Settings.
2. Select Online Run Setup and Proactive, Run Monitoring and Storage*.
3. [Optional] Enter the default output folder location.
4. Select Save.
5. On the main screen, select Start.
6. Sign into the BSSH account that was used during NextSeq 1000/2000 Integration package installation setup.
   a. Select the workgroup (if applicable).
   b. Select Next.
      Planned runs that were created in the Load to Reagent Cartridge (NextSeq 1000/2000 v2.3) step and use the Hybrid or Cloud run modes display.
7. Select the run and select Review to review the sequencing run settings.
8. Select Load and manually load the reagent cartridge and flow cell.
9. Select Sequence and the pre-run check proceeds.
   After the pre-run check passes, the sequencing run starts automatically.

Components Installed

The following sections describe the various components that are installed by default as part of this integration. These components include the following items:

- Files
- Properties

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- Reagent categories/label groups
- Reagent kits
- Control types
- Containers

Information on installed workflows, protocols, steps, and automation points is provided in the *Workflows, Protocols, and Steps* on page 1672 section.

Reagent Categories/Label Groups

- TruSeq HT adapters v2 (D7-D5)

Reagent Kits

- Resuspension Buffer (RSB)
- NextSeq 1000/2000 reagent cartridge

Container Types

- Tube
- 96-well plate
- NextSeq 1000/2000 reagent cartridge

Control Types

- PhiX v3

This integration supports the NextSeq 1000/2000 reagent cartridge with barcode provided in the format [A-Za-z]{2}[0-9]{7}-[A-Za-z0-9]{4} (eg, EC1234567-EC03).

Rules and Constraints

- The workflow configuration contains several validation checks. To make sure that the calculations work properly, it is important that you do not disable any of this validation logic. The validation checks determine:
  - Which samples, and how many, can enter each step together.
  - Which samples, and how many, can be pooled together.
- The NextSeq 1000/2000 reagent cartridge barcode must be unique. There should not be multiple NextSeq 1000/2000 reagent cartridge containers in the system with the same name.
- Reagent labels (indexes) must be unique.
- One library pool can only contain one library or control with no label (index).

- Do not manually start or complete the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3) step. This step is a fully automated step, and the SIS integration service does not update samples correctly if they have been manually started.
- Do not manually start the Demultiplexing (NextSeq 1000/2000 Sequencing v2.3) step. This step is semi-automated, and the SIS integration service does not update the demultiplexing result correctly if they have been manually started.
- For the automated run to start successfully, select Validate Run Setup and Create Planned Run in the Load to Reagent Cartridge step.

For integrations that run on Clarity LIMS v5.4, the events in the RECENT ACTIVITIES panel can reflect as the API access user instead of the user who ran the step. This is fixed in Clarity LIMS v6.0.

NextSeq 1000/2000 Integration v2.3 Validation and Troubleshooting

This section explains how to validate the installation of the Illumina NextSeq 1000/2000 Integration Package v2.3.

The validation process involves the following items:

- Running samples through the Library Prep Validation v2.2.0 workflow.
  - The workflow contains a single-step protocol that models the library prep workflow required to produce libraries tagged with index sequences. At the end of the step, these libraries are routed to NextSeq 1000/2000 Sequencing v2.3 workflow.
- Running the libraries through the NextSeq 1000/2000 Sequencing v2.3 workflow. This process validates the following information:
  - Successful sequential step advancement of samples in the following steps:
    1. Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3)
    2. Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3)
    3. AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3)
    4. Demultiplexing (NextSeq 1000/2000 Sequencing v2.3)
  - Automatic validation of run setup information before sample sheet generation or planned run creation.
  - Automated generation of a sample sheet. The sample sheet is used to start the sequencing run on NextSeq 1000/2000 Control Software (NCS) via Local run mode.
  - Automated creation of a planned run on Illumina Connected Analytics. The NextSeq 1000/2000 Control Software retrieves the planned run to start the run via Hybrid or Cloud run mode.
  - Automated tracking of the NextSeq 1000/2000 sequencing run and parsing of run statistics from BaseSpace Sequence Hub into Clarity LIMS using the SIS Integration Service.
  - Automated tracking of the secondary analysis using DRAGEN (up to BCL Convert only).
  - Parsing of demultiplexing metrics from BSSH into Clarity LIMS using SIS integration service.

Before running the validation steps below, these steps assume that the NextSeq 1000/2000 Sequencing v2.3 Integration Package is installed, and that you have imported the default Clarity LIMS configuration.

For information on how the integration works, refer to *NextSeq 1000/2000 Integration v2.3 Configuration and User Interaction* on page 1570.

Activate Workflow, Create Project, Add and Assign Samples

The following steps set up Clarity LIMS in preparation for running samples through the Library Prep Validation v2.2.0 and NextSeq 1000/2000 Sequencing v2.3 workflows.

1. On the Configuration tab, under Workflows, activate both the Library Prep Validation v2.2.0 and NextSeq 1000/2000 Sequencing v2.3 workflows.
2. On the Projects and Samples screen, create a project and add samples to it.

   Use only alphanumeric, dash, and underscore characters in the submitted sample names. Failing to do so causes sample sheet validation failure in Load To Reagent Cartridge step.

3. Assign the samples to the Library Prep Validation v2.2.0 workflow.

Library Prep Protocol: Library Prep Validation v2.2.0

This single-step protocol models the library prep required to produce libraries tagged with index sequences that are ready for the NextSeq 1000/2000 Sequencing v2.3 workflow.

Run Library Prep Validation v2.2.0 Step

1. In Lab View, locate the Library Prep Validation v2.2.0 protocol.
   Samples are queued for the Library Prep Validation v2.2.0 step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select the label groups to use.
   - TruSeq HT Adapters v2 (D7-D5) is the default label group delivered by Library Prep Validation v2.2.0 protocol.
   - For other reagent labels (indexes), manually create them in the Configuration screen under Consumables → Labels.

   For more information on how to create reagent label group, refer to the *Add and Configure Labels and Label Groups* section in the *Clarity LIMS Core Functionality* documentation.

4. Select Begin Work.
5. On the Placement screen, performing the following actions:
   a. In the Placed Samples area in the Container field, scan or enter the barcode of the destination container.
   b. Select the samples from the Samples to be Placed area, then drag them over to the container wells on the right.

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c. Select Add Labels.

6. On the Add Labels screen, the Labels to Add list is populated with labels from the TruSeq HT Adapters v2 (D7-D5) label group. This label group is the only one configured in the out-of-the-box integration. It is used by default in this step.
   a. Select labels on the left, and drag them over to the container on the right to assign them to placed samples.
   b. Select Record Details.
7. On the Record Details screen, perform the following actions:
   a. In the Sample Details table, enter values for Molarity (nM) for all the individual sample. Enter values individually or use the copy/paste functionality to enter multiple values from a spreadsheet.
      - Molarity (nM) values are used in Calculate Volume automation script in Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3) step.

      If Molarity (nM) values are not entered, the Calculate Volume automation script does not work properly.

   b. The Sequencing Instrument column indicates the instrument to be used for the run. Select NextSeq 1000/2000.
   c. Populate the fields in this column using one of the following methods:
      - To populate fields individually for each sample, select from the drop-down list in each row.
      - To populate fields all at one time, use the Copy and Paste buttons to enter multiple values from a spreadsheet (refer to the following note).

   Alternatively, select inside the table and press Ctrl + A to select all rows. At the top of the table, select Sequencing Instrument, then select NextSeq 1000/2000 from the adjacent drop-down list. Select Apply.

   If working with many samples, copy and paste to insert multiple values from a spreadsheet as follows.

      a. Select the Copy button to copy the sample information from Clarity LIMS to the clipboard.
      b. Open a blank Excel document and paste the contents of the clipboard into the spreadsheet.
      c. In the spreadsheet, enter the Molarity (nM) and Sequencing Instrument values. Select the entire spreadsheet, including the headers, (Ctrl + A) and copy (Ctrl + C) the information to the clipboard.
      d. In Clarity LIMS, select the Paste button. Paste the contents of the clipboard (Ctrl + V) into the Update Table window and then select Update to paste into the Sample Details table.
8. Select Next Steps.

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This selection triggers the Next Steps automation, which sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step successfully.

9. On the Assign Next Steps screen in the Samples table, the Next Step for all samples is prepopulated with Remove from workflow.

   Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.

10. Select Finish Step.
    On exit from the step, the Routing Script automation is triggered. This automation assigns samples to the first step of the NextSeq 1000/2000 Sequencing v2.3 workflow, which is Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3) step.

NextSeq 1000/2000 Sequencing v2.3 Protocol

Step 1: Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3)

1. In Lab View, locate the NextSeq 1000/2000 Sequencing v2.3 protocol. The samples are queued for the Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Pooling screen, perform the following actions:
   a. Create a pool by dragging samples into the Pool Creator.

Do not create more than one pool for this step. The Calculate Volumes automation in this step supports one pool only.

   b. Type a name for the pool or accept the default name (Pool #1).
   c. Select Record Details.
      On the Record Details screen, the Reagent Lot Tracking section tracks the Resuspension Buffer (RSB) lot information used in the step.
5. To add and activate additional reagent lots, perform the following steps:
   a. In a new browser tab, open an additional instance of Clarity LIMS.
   b. Navigate to the Consumables configuration screen.
   c. Select the RSB reagent kit and select New Lot.
   d. Enter the lot details and select Save.
   e. For Status of Reagent Lot, select Active.
6. In the Reagent Lot Tracking section, select from the active lots displayed in the drop-down list.
   The Step Details area contains two required fields:

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- Final Loading Concentration (pM)—The value entered in this field is the recommended final loading concentration specified in the *Illumina NextSeq 1000/2000 Sequencing System Guide*. The value depends on the library type. The default drop-down list contains the values 650, 750, 1000, and 2000. A custom value is acceptable.
- Final Loading Volume (µl)—The value in this field is the final loading volume of the pool into the reagent cartridge. The field is prepopulated with the configured default value, 24 µl, specified in the *Illumina NextSeq 1000/2000 Sequencing System Guide*. The value is editable when more volume is necessary.

7. Select Calculate Volumes.
   This selection triggers the Calculate Volumes automation. This automation calculates the volume required for each library to form a pool that has the concentration and volume specified in the step details fields.
   The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains volume information of each of the samples and RSB buffer to add to the pool. Select the file to download it, then open it in Excel.
8. In the Sample Details table, select the pool icon to view details on the pool composition.
9. Select Next Steps.
   This selection triggers the Set Next Step automation, which sets the next step for samples to ADVANCE, advancing them to the next step in the protocol. The next step is Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3).
   On the Assign Next Steps screen, the next step for samples is already set to the next step in the workflow. The next step is Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3).
10. Select Finish Step.

At the end of this step, the pool of samples automatically advances to Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3) step.

Step 2: Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3)

1. In Lab View, locate the NextSeq 1000/2000 Sequencing v2.3 protocol. The pool of samples is queued for the Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.3) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
   The Validate Single Input automation is triggered. This automation checks that there is only one container input to the step.
4. On the Placement screen, perform the following actions:
   a. Drag the pool into the NextSeq 1000/2000 Reagent Cartridge field in the Placed Samples area.
   b. Scan or type the barcode of the reagent cartridge into the NextSeq 1000/2000 Reagent Cartridge field.

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c. Select Record Details.

On exit of the Placement screen, the Validate Reagent Cartridge Barcode automation checks that the reagent cartridge barcode conforms to the barcode mask [A-Za-z]{2}[0–9]{7}-[A-Za-z0-9]{4}. If not, an error message displays.

On entry to the Record Details screen, the Retrieve Analysis Workflow Versions automation fetches the available analysis workflow versions from ICA. It then updates the preset values of both Local Analysis Workflow Versions & Cloud Analysis Workflow Versions field.

5. On the Record Details screen, the Reagent LotTracking section tracks the NextSeq 1000/2000 Reagent Cartridge lot information used in the step. Follow the steps in Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.3) step if you must add a lot.
6. In the Reagent Lot Tracking section, select from the active lots displayed in the drop-down list.
7. The fields displayed on the Record Details screen are used to create planned run and generate the sample sheet file.
   - Run Name—Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.
   - Instrument Type—Select from preset options (NextSeq 1000 or NextSeq 2000).
   - Run Mode—Select from preset options: Local, Hybrid, Cloud. For more information on the different run modes, refer to *Run Mode Details* on page 1612.
   - Paired End—Select from preset options (True or False).
   - Read 1 Cycles—Select from preset options (301, 151, 101, 51) or type a custom value.
   - Read 2 Cycles—Select from preset options (301, 151, 101, 51) or type a custom value.
   - Index Reads—Select from preset options (No Index, Single Index, Dual Index).
   - Index Read 1—Select from preset options (0, 6, 8) or type a custom value.
   - Index Read 2—Select from preset options (0, 6, 8) or type a custom value.
   - Analysis Workflow—Read only. This field is set to GenerateFASTQ.
   - Local Analysis Workflow Versions—[Optional] (default is None). Only mandatory when Run Mode = Local/Hybrid and Analysis Workflow = GenerateFASTQ. Select according to the DRAGEN version installed on the instrument.
   - Cloud Analysis Workflow Versions—[Optional] (default is None). Only mandatory when Run Mode = Cloud and Analysis Workflow = GenerateFASTQ. Select the cloud DRAGEN version to be used.
   - FASTQ Compression Format:
     - If Run Mode = Local/Hybrid, Analysis Workflow = GenerateFASTQ, and Local Analysis Workflow Version >= 3.7.4, select gzip or DRAGEN.
     - If Run Mode = Cloud, Analysis Workflow = GenerateFASTQ, and Local Analysis Workflow Version >= 3.7.4, field is set to gzip automatically.

    - Otherwise, select None.
  - Adapter Sequence Read 1—[Optional] Enter the Read 1 adapter sequence of the index adapter kit.
  - Adapter Sequence Read 2—[Optional] Enter the Read 2 adapter sequence of the index adapter kit.
  - Barcode Mismatches Index 1—[Optional] Select from preset options (0, 1, 2). Leave it blank if you are unsure.
  - Barcode Mismatches Index 2—[Optional] Select from preset options (0, 1, 2). Leave it blank if you are unsure.
  - Override Cycles—[Optional] String used to specify UMI cycles and mask out cycles of a read (eg, N1Y150;I8;I7N1;Y141U10). Leave it blank if you are unsure.
  - Instructions — Read only.
8. On the Record Details screen, select Validate Run Setup and Create Planned Run.
   This selection triggers the automation script, which does the following actions:
   - Validates the parameters entered on the Record Details screen.
   - Create the planned run and send it to ICA if Run Mode is Hybrid or Cloud.
   - Generates the sample sheet and attaches it to the placeholder in the Files area of the Record Details screen for all Run Mode types.
9. Select Next Steps.
   On the Assign Next Steps screen, the next step for samples is set to the next step in the workflow. The next step is AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3).
10. Select Finish Step.

At the end of this step, the pool of samples automatically advances to and is queued for the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3) step. The sequencing run is ready to be started. For details on how to start the sequencing run for different run modes, refer to *NextSeq 1000/2000 Integration v2.3 Configuration and User Interaction* on page 1570.

Run Mode Details

The run mode applies to all runs and determines how the run and analysis parameters are configured and how to analyze data.

- Local Mode—NextSeq 1000/2000 Integration service generates the sample sheet. Run/Analysis configuration is imported manually into NextSeq 1000/2000 instrument via a sample sheet to start the run. The downstream secondary analysis is done using the onboard DRAGEN module.
- Hybrid Mode—NextSeq 1000/2000 Integration service sends the run/analysis configuration to Illumina Connected Analytics. Run/Analysis configuration is downloaded to NextSeq 1000/2000 instrument via ICA to start the run. The downstream secondary analysis is done using the onboard DRAGEN module.

- Cloud Mode—NextSeq 1000/2000 Integration service sends the run/analysis configuration to ICA. Run/Analysis configuration is downloaded to NextSeq 1000/2000 instrument via ICA to start the run. The downstream secondary analysis is performed in the cloud.

Step 3: AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3)

This step is fully automated.

Do not add samples to the Ice Bucket or start or complete the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3) step. The integration does this action automatically.

The integration starts the step automatically and data from the run is parsed back to Clarity LIMS. No user interaction is required. However, you can open and review the various stages of the step in Clarity LIMS. Do not perform any action when reviewing the data.

Read summary metrics are recorded for the library pool in the Step Details section and the Sample Details table.

Values are populated in the following master step fields:

Run Name	Run Status	Output Folder
Current Read	Current Cycle	Secondary Analysis Workflow
Flow Cell ID	Flow Cell Lot Number	Reagent Cartridge ID
Reagent Cartridge Lot Number	Instrument Platform	Instrument Type
Instrument ID	Instrument Control Software Version	RTA Version
Sequencing Log		

The summary metrics (per run level) populate in the following global fields.

- % Bases >=Q30 R1
- % Bases >=Q30 R2
- % Error Rate R1
- % Error Rate R2
- Yield (Gb) R1
- Yield (Gb) R2
- Reads PF R1
- Reads PF R2
- %PF R1

- %PF R2
- % Aligned R1
- % Aligned R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- Cluster Density R1
- Cluster Density R2

At the end of this step, the pool of samples automatically advances to (and queues for) the Demultiplexing (NextSeq 1000/2000 Sequencing v2.3) step.

Step 4: Demultiplexing (NextSeq 1000/2000 Sequencing v2.3)

This step is semi-automated.

For the demultiplexing information in the Sample Details table to display correctly, the following conditions must be met:

- The DRAGEN version must be earlier than v3.9.3.
- The samples in the run must not repeat.
- The run must be for the single lane flow cell (P1 or P2).

Do not add samples to the Ice Bucket or start or complete the step. The integration completes these actions automatically.

The integration starts the step automatically and demultiplexing data from the GenerateFASTQ secondary analysis is parsed back to Clarity LIMS. Review the data parsed and assign QC, depending on the criteria set, and complete the step.

In the Record Details screen, perform the following actions:

1. Review demultiplexing data.
   - Demultiplexing metrics are recorded for the library pool in the Sample Details table. Metrics include columns for # Reads, # Perfect Index Reads, # One Mismatch Index Reads, and # of >= Q30 Bases (PF).
   - The `Demultiplex_stats.csv` file generated by the Dragen onboard or Cloud analysis module is stored under the Files section.

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2. Assign QC flags to all the individual sample. There are two ways of doing this step.
   a. Manually assign QC flags through the QC column in Sample Details table.
   b. Automatically assign QC flag by running Assign QC flags automation. This option is for scenarios where a huge number of libraries are involved.

   In the Step Details section, the following step fields are visible. (N is the number of criteria. You can use one or more criteria.)
   - Criteria N - Source Data Field—Select from preset options eg # Reads.
   - Criteria N - Operator—Select from preset options, for example, >= (greater than or equal to).
   - Criteria N - Threshold Value—Enter the desired threshold value.
3. After filling up the criteria fields, select Assign QC flags. This selection triggers the automation script, which loops through each library in the pool and apply QC flag base on the criteria set previously.
   This automation also generates an AssignQC Result file under Files section.
4. Select Next Steps.
   On the Assign Next Steps screen, the Next Step field for all samples is prepopulated with Mark protocol as complete.
5. Select Finish Step.

At this point, the whole NextSeq 1000/2000 Integration workflow is fully validated.

Troubleshooting

If an automation trigger does not appear to run its corresponding scripts, refer to *Troubleshooting Automation* in the Clarity LIMS API documentation.

If the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3) step starts but does not finish, complete the following steps:

1. Log in to Clarity LIMS using the default user account and use one of the following methods to open the step in Clarity LIMS:
   - Method 1: In Lab View, find the step in the Recent Activities pane.
   - Method 2: Search for the step in Clarity LIMS using reagent cartridge barcode as the search term.
2. On the Record Details screen, locate the Sequencing Log field.
   The multiline text field contains logging information.

If you cannot reach the Record Details screen, or if the Sequencing Log field does not contain enough information to resolve the issue, contact the Clarity LIMS support team. Supply the relevant information from the troubleshooting steps already performed.

For integrations that run on Clarity LIMS v5.4, the events in the RECENT ACTIVITIES panel can reflect as the API access user instead of the user who ran the step. This is fixed in Clarity LIMS v6.0.

NextSeq 1000/2000 Integration v2.3 Release Notes

These Release Notes describe the key changes to software components for the Clarity LIMS NextSeq 1000/2000 Integration Package v2.3.

If upgrading from a version prior to v2.1, review the release notes for v2.1 for a list of features and bug fixes introduced in that version.

Compatibility

This version of the integration package is compatible with the following software:

- Clarity LIMS v5.3 and later (up to v6.0 only)
- NextSeq 1000/2000 Instrument Control Software (NCS) v1.5.0

New Features

Features provided with this release of the integration package are as follows:

- Supports NCS v1.5.0
- Supports new P1 and P2 long read reagent cartridges
- Updates vault key names
- Bug fixes

Defect Repairs

- Fixed the logic to support the new `RunParameters.xml` format introduced in NCS v1.5.0.
- Removed the unsupported None option for the Analysis Workflow field in the Load to Reagent Cartridge step of the NextSeq 1000/2000 workflow. All planned runs that are created will have the analysis configuration associated.

Known Issues

- Sequencing run error triggered by disk space full on instrument will cause `RunParameters.xml` to be empty and the run event will not be able to be processed on Clarity. This error is unlikely to occur as pre-run checks on the instrument check for sufficient disk space before run is started.

Limitations

For the demultiplexing information in the Sample Details table to display correctly, the following conditions must be met:

- The DRAGEN version must be earlier than v3.9.3.
- The samples in the run must not repeat.
- The run must be for the single lane flow cell (P1 or P2).

NextSeq 1000/2000 Integration v2.2 Configuration and User Interaction

Compatible with Illumina cloud hosted deployments only

The Illumina NextSeq 1000/2000 Integration Package v2.2 supports the integration of Clarity LIMS to Illumina NextSeq 1000/2000 sequencing systems.

For instructions on validating and troubleshooting the NextSeq 1000/2000 Integration, see *NextSeq 1000/2000 Integration v2.2 Validation and Troubleshooting* on page 1651.

The configuration provided in this integration has been established to support NextSeq 1000/2000 lab processes. Any configuration changes to protocols or workflows—including renaming protocols, steps, and fields—could break the process.

Overview

The integration includes the following features:

- Preconfigured NextSeq 1000/2000 Sequencing v2.1 workflow that maps to lab protocols and instrument runs.
- Preconfigured protocols:
    - Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.1)
    - Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.1)
    - AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.1)
    - Demultiplexing (NextSeq 1000/2000 Sequencing v2.1)
- Automatic validation of run setup information before sample sheet generation or planned run creation.
- Automated generation of a sample sheet. The sample sheet is used to start the sequencing run on NextSeq 1000/2000 Control Software via Local run mode.
- Automated creation of a planned run on Illumina Connected Analytics v1.0. The NextSeq 1000/2000 Control Software retrieves and starts the planned run via Hybrid or Cloud run mode.
- Automated tracking of the NextSeq 1000/2000 sequencing run and parsing of run statistics from BaseSpace Sequence Hub into Clarity LIMS using SIS integration service.
- Automated tracking of the secondary analysis using DRAGEN (up to BCL Convert only) and parsing of demultiplexing metrics from BaseSpace Sequence Hub into Clarity LIMS using SIS integration service.
- [Optional] Preconfigured Library Prep Validation v2.0.4 workflow used for validation purposes only. The workflow contains a single-step protocol that models the library prep workflow required

to produce libraries tagged with index sequences. At the end of the step, these libraries are routed to NextSeq 1000/2000 Sequencing v2.1 workflow. See *NextSeq 1000/2000 Integration v2.2 Validation and Troubleshooting* on page 1651.

Information on these features is provided in the following sections.

Prerequisites and Assumptions

The Illumina NextSeq 1000/2000 Integration Package v2.2 is compatible with Illumina cloud hosted deployments only.

It is assumed that samples entering the NextSeq 1000/2000 Sequencing v2.1 workflow have gone through library preparation and quantification processes. Before they are assigned to the workflow, samples have completed the following actions:

- Samples have been accessioned into Clarity LIMS.
- Samples have been run through QC and library prep.
- Samples have the Molarity (nM) global field set to some value.
  - The Calculate Volumes automation in the Library Pooling and Dilution step requires a value in the Molarity (nM) global field.

For more information on sample accessioning, refer to *Sample Accessioning* and *Upload and Modify Samples* in the Getting Started section of the Clarity LIMS Core Functionality documentation.

You can assign samples to workflows automatically, using a routing script, or manually—from the Projects & Samples dashboard. Refer to *Assign and Process Samples* in the Clarity LIMS Core Functionality documentation.

Workflows, Protocols, and Steps

The Illumina NextSeq 1000/2000 Integration Package v2.2 includes two workflows:

- NextSeq 1000/2000 Sequencing v2.1

  Samples are routed to this protocol after they have gone through Library Prep Validation v2.0.4 (or any other library prep workflow). Samples are pooled, diluted to final loading concentration, and added to a reagent cartridge in preparation for the sequencing run.
- [Optional] Library Prep Validation v2.0.4 - recommended for validation purposes.

The following table lists and describes the protocols and steps included in these workflows.

The workflow configuration includes a global field named Illumina Universal Sample Identifier. This text field is optional and reserved for a future feature. The field value is not required for this integration.

Table 334 Illumina NextSeq 1000/2000 Integration Package v2.2 Protocols and Steps

Protocol Name	Protocol Purpose	Steps Included in Protocol
Library Prep Validation v2.0.4 Workflow		
Library Prep Validation v2.0.4	Included for validation purposes only, this protocol models the library prep steps required to advance samples to the NextSeq 1000/2000 Sequencing v2.1 workflow. The protocol contains a single step—Library Prep Validation v2.0.4. At the end of this step, a routing script sends the samples to the first step of the NextSeq 1000/2000 Sequencing v2.1 workflow. The first step is Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.1).	1. Library Prep Validation v2.0.4
NextSeq 1000/2000 Sequencing v2.1 workflow		

Protocol Name	Protocol Purpose	Steps Included in Protocol
NextSeq 1000/2000 Sequencing v2.1	This protocol models the lab processes of starting a NextSeq 1000/2000 sequencing run. Details of the steps: 1. Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.1) a. Samples are pooled and diluted to the final loading concentration with the help of the Calculate Volume script. 2. Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.1) a. The library pool from step 1 is then ready to be loaded to the NextSeq 1000/2000 reagent cartridge. b. Run and analysis information is validated. c. Sample sheet is generated and/or planned run is created. 3. AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.1) a. This step is a fully automated step that is started and completed automatically after the sequencing run is started and completed on the instrument side. b. All the metadata (eg, run configuration, primary run metrics, etc.) of the sequencing run are recorded automatically. Do not add samples to the Ice Bucket or start or complete the step. The integration completes these actions automatically. c. If GenerateFastQ secondary analysis is not planned for the run, samples complete the	1. Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.1) 2. Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.1) 3. AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.1) 4. Demultiplexing (NextSeq 1000/2000 Sequencing v2.1)

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Validation Workflow

The Library Prep Validation v2.0.4 workflow allows for validation of the system after installation is complete. For details, see *NextSeq 1000/2000 Integration v2.2 Validation and Troubleshooting* on page 1651*NextSeq 1000/2000 Integration v2.2 Validation and Troubleshooting* on page 1651

Step Automations

Automation details are provided in the sections following the table.

For details on the validation workflow, see *NextSeq 1000/2000 Integration v2.2 Validation and Troubleshooting* on page 1651.

Table 335 NextSeq 1000/2000 Sequencing v2.1 Workflow Step Automations

Protocol	Step	Automation
NextSeq 1000/2000 Sequencing v2.1	Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.1)	• Calculate Volumes • Set Next Step
	Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.1)	• Set Next Step • Validate Single Input • Validate Reagent Cartridge Barcode • Retrieve Analysis Workflow Versions • Validate Run Setup and Create Planned Run
	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.1)	• Set Next Step
	Demultiplexing (NextSeq 1000/2000 Sequencing v2.1)	• Assign Demultiplex QC flags • Set Next Step

Terminology

The Illumina NextSeq 1000/2000 Integration Package v2.2 supports Clarity LIMS v5.3 and later.

As of Clarity LIMS v5, the term user-defined field (UDF) has been replaced with custom field in the user interface. However, the API resource is still called UDF.

There are two types of custom fields:

- Master step fields—Configured on master steps. Master step fields only apply to the following:
  - The master step on which the fields are configured.
  - The steps derived from those master steps.

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- Global fields—Configured on entities (eg, submitted sample, derived sample, measurement, etc.). Global fields apply to the entire Clarity LIMS system.

Step 1: Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.1)

In this step, pooled samples are diluted by the addition of RSB. Manually create a working pool based on the final loading concentration required.

Only create one pool per step for the Calculate Volume automation script to work properly.

Calculate Volumes Automation

Select the Calculate Volumes button on the Record Details screen to trigger this automation automatically, which does the following actions:

- Sets RSB Volume for Pool (µl) field value to 24 for calculation purpose of the 2 nM intermediate library pool.
- Copies the Final Loading Concentration (pM) from step inputs to step outputs.

```
-exp 'step.::RSB Volume for Pool (µl):: = 24; output.::Final Loading
Concentration (pM):: = step.::Final Loading Concentration (pM)::'
```

- Calculate per sample volume required for each library to make the 2 nM intermediate library pool (24ul in volume).

```
-exp 'input.::Per Sample Volume (µl):: = ( 48/ input.::Molarity (nM):: );
step.::RSB Volume for Pool (µl):: -= input.::Per Sample Volume (µl)::'
```

- Calculate the volume required of the 2 nM library pool to be diluted further to the Final Loading Concentration (pM) with the Final Loading Volume (µl).

```
-exp 'step.::Library Pool Volume (µl):: = (step.::Final Loading Concentration
(pM):: * step.::Final Loading Volume (µl)::/2000); output.::RSB Volume (µl):: =
step.::Final Loading Volume (µl):: - step.::Library Pool Volume (µl)::'
```

- Uses the `NextSeq1K2K_Pool1.csv`, `NextSeq1K2K_Pool2.csv`, and `NextSeq1K2K_Pool3.csv` template files to generate a single CSV file containing information about the pool and the samples it contains. The generated file is stored in the Calculation File placeholder, in the Files section, for download.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} \
   script:driver_file_generator \
      -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NextSeq1K2K_
      Pool1.csv \
      -o 1.csv \
   script:driver_file_generator \
```


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```
      -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NextSeq1K2K_
      Pool2.csv \
      -o 2.csv \
   script:driver_file_generator \
      -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NextSeq1K2K_
      Pool3.csv \
      -o 3.csv \
&& cat 1.csv 2.csv 3.csv > {compoundOutputFileLuid0}.csv
```

Set Next Step Automation

Automatically triggered on exit of the Record Details screen, this automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol. The next step is Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.1):

```
nextStep = ::ADVANCE::
```

Master Step Fields

There are four fields defined on the Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.1) step.

Table 336 Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.1) Master Step Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Final Loading Concentration (pM)	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 650 • 750 • 1000 • 2000
Final Loading Volume (µl)	Numeric	• Required: Yes • Read Only: No	• Default: 24

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Library Pool Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Hidden ⚠ This field is used by the Calculate Volumes automation. Removing, renaming, or modifying the field will break the automation.
RSB Volume for Pool (µl)	Numeric	• Required: No • Read Only: Yes	• Hidden ⚠ This field is used by the Calculate Volumes automation. Removing, renaming, or modifying the field will break the automation.

Global Fields

The following table lists the global fields that are configured to display on the Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.1) step.

Table 337 Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.1) Global Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Final Loading Concentration (pM)	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 225 • 400 • Decimal Places Displayed: 0
RSB Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2

User Interaction

Refer to *NextSeq 1000/2000 Integration v2.2 Validation and Troubleshooting* on page 1651 for details.

Step 2: Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.1)

In this step, scan the reagent cartridge barcode into Clarity LIMS, then manually place the working pool into the reagent cartridge for the NextSeq 1000/2000 run. This step validates the run setup and analysis information and generates the sample sheet file and/or creates a planned run on Illumina Connected Analytics, depending on the Run Mode selected.

Validate Single Input Automation

Automatically triggered at the beginning of the step, this automation checks that there is only one container input to the step.

```
script:validateSampleCount -min 1 -max 1
```

Validate Reagent Cartridge Barcode Automation

Automatically triggered on exit of the Placement screen, this automation does the following action:

- Validates the reagent cartridge barcode to make sure it conforms to the barcode mask [A-Za-z]{2}[0-9]{7}-[A-Za-z0-9]{4}:

```
if (!output.container.name.matches( ::[A-Za-z]{2}[0-9]{7}-[A-Za-z0-9]{4}:: ) )
{fail ( ::Invalid Reagent Cartridge Barcode. Please verify and try again.:: ) }
```

Retrieve Analysis Workflow Versions Automation

Automatically triggered upon entry of the Record Details screen, this automation:

Fetches the available analysis workflow versions from ICA and updates the preset values of both Local Analysis Workflow Versions & Cloud Analysis Workflow Versions fields.

```
/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/nextseq1k2k-
automation.jar script:retrieve_analysis_workflow_versions -i {stepURI:v2} -u
{username} -p {password} -l {compoundOutputFileLuid2}
```

Do not modify or disable this automation script as it breaks the integration.

Validate Run Setup and Create Planned Run Automation

Automatically triggered when a button on the Record Details screen is selected, this automation does the following actions:

- Validates the parameters entered on the Record Details screen. These parameters are used to set up the run and generate the sample sheet file and/or create the planned run on ICA.
  - Run Name can only contain alphanumeric, dash, underscore, or period characters. Spaces are not permitted.

```
if (!step.::Run Name::.matches(::[a-zA-Z0-9-_.]+::))
{ fail(::Run Name contains prohibited characters. Allowed characters are: a-z,
A-Z, 0-9, -, _, and .::) }
```

  - Run Name must not exceed 255 characters.

```
if (step.::Run Name::.length() > 255) { fail(::Run Name shall not exceed 255
characters.::) };
```

- Checks the Index Reads, Index Read 1, and Index Read 2 field values.
  - If Index Reads is No Index, Index Read 1 and Index Read 2 values must be zero (error results if it is otherwise).
  - If Index Reads is Single Index, Index Read 1 value must be greater than zero and Index Read 2 values must be zero (error results if it is otherwise).
  - If Index Reads is Dual Index, Index Read 1 and Index Read 2 values must be greater than zero (error results if it is otherwise).

```
if (step.::Index Reads:: == ::No Index::)
{ if (step.::Index Read 1:: != 0 || step.::Index Read 2:: != 0) { fail(::Index
Read 1 and Index Read 2 must be 0 if the Index Reads is No Index.::) } }
else
{ if (step.::Index Reads:: == ::Single Index::)
{ if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) { fail(::Index
Read 1 must be greater than 0 and Index Read 2 must be 0 if the Index Reads is
Single Index.::) } }
else
```


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```
{ if (step.::Index Read 1:: == 0 || step.::Index Read 2:: == 0) { fail(::Index Read 1 and Index Read 2 must be greater than 0 if the Index Reads is Dual Index.::) } } }
```

- Checks the Paired End, Read 2 Cycles, and Index Read 2 field values.
  - If Paired End is set to True and Read 2 Cycles value is zero, an error is generated.
  - If Paired End is set to False and Read 2 Cycles or Index Read 2 values are greater than zero, an error is generated.

```
if (step.::Paired End::.toBoolean())
{ if (step.::Read 2 Cycles:: == 0) { fail(::Read 2 Cycles must not be zero if it is Paired End read.::) }}
else
{if (step.::Read 2 Cycles:: != 0 || step.::Index Read 2:: != 0) {fail(::Read 2 Cycles and Index 2 Cycles must be 0 if it is not Paired End Read.:: )}}
```

- Checks the Adapter Sequence Read 1 and Adapter Sequence Read 2 field values.
  - Adapter Sequence Read 1 and Adapter Sequence Read 2 can only contain ACTG+ characters.

```
if (step.hasValue(::Adapter Sequence Read 1::) && !step.::Adapter Sequence Read 1::.matches(::[ACTG+]+::)) { fail(::Adapter Sequence Read 1 contains prohibited characters. Allowed characters are: ACTG+::) };
if (step.hasValue(::Adapter Sequence Read 2::) && !step.::Adapter Sequence Read 2::.matches(::[ACTG+]+::)) { fail(::Adapter Sequence Read 2 contains prohibited characters. Allowed characters are: ACTG+::) }
```

- Checks Override Cycles field value.
  - Override Cycles can only contain Y, N, I, U, 0–9, and semicolon characters.

```
if (step.hasValue(::Override Cycles::) && !step.::Override Cycles::.matches(::[YNIU0-9;]+::))
{ fail(::Override Cycles contains prohibited characters. Allowed characters are: Y, N, I, U, 0-9 and ;. Example: N1Y150;I8;I7N1;Y141U10.::) }
```

- Sets Cloud Analysis Workflow Versions, Local Analysis Workflow Versions and FASTQ Compression Format to None when Analysis Workflow is "None".

```
if (step.::Analysis Workflow:: == ::None::) { step.::Cloud Analysis Workflow Versions:: = ::None::; step.::Local Analysis Workflow Versions:: = ::None::; step.::FASTQ Compression Format:: = ::None::; }
```

- When Analysis Workflow is GenerateFASTQ and Run Mode is Cloud:
  - Set Local Analysis Workflow Versions to None.
  - If Cloud Analysis Workflow Versions is None throw the following error message: Invalid option selected for Cloud Analysis Workflow Versions field.

```
if (step.::Analysis Workflow:: == ::GenerateFASTQ:: && step.::Run Mode:: ==
::Cloud::) { step.::Local Analysis Workflow Versions:: = ::None::; if
(step.::Cloud Analysis Workflow Versions:: == ::None::) { fail(::Invalid option
selected for Cloud Analysis Workflow Versions field.::); }};
```

  - Due to automation script limitation, a validation rule resides in the backend service to perform the following action: If Cloud Analysis Workflow Versions is 3.7.4 or later, set FASTQ Compression Format to gzip.

- When Analysis Workflow is GenerateFASTQ and Run Mode is Local or Hybrid:
  - Set Cloud Analysis Workflow Versions to None.
  - If Local Analysis Workflow Versions is None throw an error Invalid option selected for Local Analysis Workflow Versions field.

```
if (step.::Analysis Workflow:: == ::GenerateFASTQ:: && (step.::Run Mode:: ==
::Local:: || step.::Run Mode:: == ::Hybrid::)) { step.::Cloud Analysis Workflow
Versions:: = ::None::; if (step.::Local Analysis Workflow Versions:: ==
::None::) { fail(::Invalid option selected for Local Analysis Workflow Versions
field.::); }};
```

  - Due to automation script limitation, a validation rule resides in the backend service to perform the following action: If Local Analysis Workflow Versions is 3.7.4 or above, throw an error if FASTQ Compression Format is "None".

- Generates the sample sheet and/or creates the planned run on ICA.
  - Sample sheet is attached to the step.

```
/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/nextseq1k2k-
automation.jar
script:generate_v2_sample_sheet -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid2} -c {compoundOutputFileLuid0}
```

  - There is backend validation of the sample sheet content. This validation makes sure that the sample sheet is valid to set up a Local Mode run or is ready to be consumed by DRAGEN applications. Refer to *NextSeq 1000/2000 Integration v2.2 Validation and Troubleshooting* on page 1651 for submitted sample name character restrictions.

Set Next Step Automation

Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol—AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.1).

```
nextStep = ::ADVANCE::
```

Master Step Fields

There are 24 fields defined on the Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.1) step. These fields are required for sample sheet generation and planned run creation on ICA.

Table 338 Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.1) Master Step Fields Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Run Name	Text	• Required: Yes • Read Only: No	Not applicable
Instrument Type	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• NextSeq1000 • NextSeq2000
Run Mode	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• Local • Hybrid • Cloud
Paired End	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• True • False
Read 1 Cycles	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 151 • 101 • 51
Read 2 Cycles	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 151 • 101 • 51
Index Reads	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• No Index • Single Index • Dual Index
Index Read 1	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 0 • 6 • 8

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Index Read 2	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 0 • 6 • 8
Analysis Workflow	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• GenerateFASTQ • None
Adapter Sequence Read 1	Text	• Required: No • Read Only: No	Not applicable
Adapter Sequence Read 2	Text	• Required: No • Read Only: No	Not applicable
Barcode Mismatches Index 1	Numeric Dropdown	• Required: No • Read Only: No • Custom Entries: No	• 0 • 1 • 2
Barcode Mismatches Index 2	Numeric Dropdown	• Required: No • Read Only: No • Custom Entries: No	• 0 • 1 • 2
Override Cycles	Text	• Required: No • Read Only: No	Not applicable
Cloud Run ID	Text	• Required: No • Read Only: Yes	• Hidden - Used in creating planned run in ICA. Do not delete, rename, or modify. These actions will break the integration.

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Use Custom Index Read 1 Primer	Toggle Switch	• Required: No • Read Only: No	• Hidden Custom primer option is currently not supported. Do not enable as it will break the integration.
Use Custom Index Read 2 Primer	Toggle Switch	• Required: No • Read Only: No	• Hidden Custom primer option is currently not supported. Do not enable as it will break the integration.
Use Custom Read 1 Primer	Toggle Switch	• Required: No • Read Only: No	• Hidden Custom primer option is currently not supported. Do not enable as it will break the integration.

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Use Custom Read 2 Primer	Toggle Switch	• Required: No • Read Only: No	• Hidden Custom primer option is currently not supported. Do not enable as it will break the integration.
Instructions	Multi-Line Text	• Required: No • Read Only: Yes	• Default: If GenerateFASTQ analysis is required, select the DRAGEN version in Cloud Analysis Workflow Versions (for Cloud run mode) or Local Analysis Workflow Versions (for Local/Hybrid run mode) custom fields.
Local Analysis Workflow Versions	Text Dropdown	• Required: No • Read Only: No • Custom Entries: No	• None (default value) Do not delete, rename, or modify. These actions will break the integration.

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Cloud Analysis Workflow Versions	Text Dropdown	• Required: No • Read Only: No • Custom Entries: No	• None (default value) Do not delete, rename, or modify. These actions will break the integration.
FASTQ Compression Format	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• GZIP (default value) • DRAGEN • None

User Interaction

Refer to *NextSeq 1000/2000 Integration v2.2 Validation and Troubleshooting* on page 1651 for details.

Step 3: AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.1)

This step is fully automated.

The integrations start and complete the step automatically and data from the run is parsed back to Clarity LIMS. No user interaction is required. In this step, the pooled samples in the reagent cartridge are sequenced on the NextSeq 1000/2000 instrument.

Do not add samples to the Ice Bucket or start or complete the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.1) step. The integration does this step automatically.

Set Next Step Automation

The Set Next Step automation is the only automation configured on this step. The automation automatically sets the samples to advance to the next step—Demultiplexing (NextSeq 1000/2000 Sequencing v2.1).

```
nextStep = ::ADVANCE::
```

Master Step Fields

There are 24 fields defined on the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.1) step. These fields are used to display the run status and sequencing run and analysis configuration parsed from the `RunParameters.xml` file of the sequencing run.

Do not modify the name of any of the master step fields. This action causes the integration to break.

Run Parameters and Corresponding Clarity LIMS Step Fields

The following table shows how some of the step fields map to the fields on the `RunParameters.xml` file, and whether the field is visible on the Record Details screen.

Master Step Field	RunParameters.xml Field	On Record Details Screen
Current Cycle	Calculated based on CompletedCycles Field	Visible
Current Read	Calculated based on CompletedCycles field against PlannedCycles	Visible
Flow Cell ID	FlowCellSerialNumber	Visible
Flow Cell Lot Number	FlowCellLotNumber	Visible
Instrument Control Software Version	ApplicationVersion	Visible
Instrument ID	InstrumentSerialNumber	Visible
Output Folder	OutputFolder	Visible
Reagent Cartridge ID	CartridgeSerialNumber	Visible
Reagent Cartridge Lot Number	CartridgeLotNumber	Visible
RTA Version	RtaVersion	Visible
Run Name	ExperimentName	Visible
Secondary Analysis Workflow	SecondaryAnalysisWorkflow	Visible

Master Step Field	RunParameters.xml Field	On Record Details Screen
Flow Cell Mode	FlowCellMode	Hidden Do not delete, rename, or modify. These actions will break the integration.
Instrument Run ID	Derived from OutputFolder	Hidden Do not delete, rename, or modify. These actions will break the integration.
Run End Time	RunEndTime	Hidden Do not delete, rename, or modify. These actions will break the integration.
Run Start Time	RunStartTime	Hidden Do not delete, rename, or modify. These actions will break the integration.
Secondary Analysis Mode	SecondaryAnalysisMode	Hidden Do not delete, rename, or modify. These actions will break the integration.
Secondary Analysis Platform Version	SecondaryAnalysisPlatformVersion	Hidden Do not delete, rename, or modify. These actions will break the integration.

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Master Step Field	RunParameters.xml Field	On Record Details Screen
SkipObdd	SkipObdd	Hidden Do not delete, rename, or modify. These actions will break the integration.

Additional Master Step Fields and Values

The following table shows how the other step fields derive their values, and whether the step field is visible on the Record Details screen.

Master Step Field	RunParameters.xml Field	On Record Details Screen
Instrument Platform	NextSeq 1000/2000 Constant value	Visible
Instrument Type	One of the following: • NextSeq1000 • NextSeq2000 Determined from Instrument Type step field from previous Load to Reagent Cartridge step.	Visible
Run Status	One of the following: • RunStarted • RunCompletedSuccessfully • RunAbortedByUser • RunErroredOut Set by the SIS integration service*	Visible
Sequencing Log	Filled in by the SIS integration service* as the sequencing run proceeds	Visible
BaseSpace Run ID	Received from SQS message in the SIS integration service	Hidden Do not delete, rename, or modify. These actions will break the integration.

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*For information on how the integration works, see *Run Status, Primary Metrics and Analysis Results Parsing and Recording* on page 1642.

Global Fields

The following global fields are used to capture the run metrics in Clarity LIMS.

- % Bases >=Q30 R1
- % Bases >=Q30 R2
- % Error Rate R1
- % Error Rate R2
- Yield (Gb) R1
- Yield (Gb) R2
- Reads PF R1
- Reads PF R2
- %PF R1
- %PF R2
- % Aligned R1
- % Aligned R2
- % Phasing R1
- % Phasing R2
- % PrePhasing R1
- % PrePhasing R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- Cluster Density R1
- Cluster Density R2

Step Completion

At the end of the step, the pool of samples undergoes one of the following actions:

- If the Analysis Workflow field in Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.1) step is set to GenerateFASTQ, the pool automatically advances to and is queued for the Demultiplexing (NextSeq 1000/2000 Sequencing v2.1) step.

- If the Analysis Workflow field in Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.1) step is set to None, the pool automatically completes the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.1) step and is removed from the workflow.

Step 4: Demultiplexing (NextSeq 1000/2000 Sequencing v2.1)

This step is a semiautomated step.

The integration starts the step automatically and demultiplexing data from the GenerateFASTQ secondary analysis is parsed back to Clarity LIMS. The lab scientist reviews the demultiplexing result parsed into Clarity LIMS, assigns QC flags, and completes the step.

For the demultiplexing information in the Sample Details table to display correctly, the following conditions must be met:

- The DRAGEN version must be earlier than v3.9.3.
- The samples in the run must not repeat.
- The run must be for the single lane flow cell (P1 or P2).

Do not add samples to the Ice Bucket or start or complete the step. The integration completes these actions automatically.

Automations

The following automations are configured on the step:

- Assign Demultiplexing QC flags—Automatically assigns QC flags based on the criteria set on the step fields.
- Set Next Step—Automatically sets the next step to Advance and the samples complete the protocol.

Master Step Fields

The following table lists the 12 master step fields that are configured on the Demultiplexing (NextSeq 1000/2000 Sequencing v2.1) master step.

Table 339 Master Step Field Configuration for Demultiplexing (NextSeq 1000/2000 Sequencing v2.1) Step

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Criteria 1 - Source Data Field	Text Dropdown	• Required: No • Read Only: No • Custom Entries: Yes	• # Reads • # Perfect Index Reads • # One Mismatch Index Reads • # of >= Q30 Bases (PF)
Criteria 2 - Source Data Field	Text Dropdown	• Required: No • Read Only: No • Custom Entries: Yes	• # Reads • # Perfect Index Reads • # One Mismatch Index Reads • # of >= Q30 Bases (PF)
Criteria 1 - Operator	Text Dropdown	• Required: No • Read Only: No • Custom Entries: Yes	• >= • <= • = • !=
Criteria 2 - Operator	Text Dropdown	• Required: No • Read Only: No • Custom Entries: Yes	• >= • <= • = • !=
Criteria 1 - Threshold Value	Numeric	• Required: No • Read Only: No	• Valid integer value
Criteria 2 - Threshold Value	Numeric	• Required: No • Read Only: No	• Valid integer value
Log	Multiline Text	• Required: No • Read Only: Yes	Not applicable

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Analysis Completed Time	Text	• Required: No • Read Only: Yes	Hidden Do not delete, rename, or modify. These actions will break the integration.
Analysis Started Time	Text	• Required: No • Read Only: Yes	Hidden Do not delete, rename, or modify. These actions will break the integration.
Analysis Status	Text	• Required: No • Read Only: Yes	Hidden Do not delete, rename, or modify. These actions will break the integration.
BSSH Analysis ID	Text	• Required: No • Read Only: Yes	Hidden Do not delete, rename, or modify. These actions will break the integration.

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Reagent Cartridge ID	Text	• Required: No • Read Only: Yes	• Hidden • Used for parsing demultiplexing results Do not delete, rename, or modify. These actions will break the integration.

Global Fields

There are five fields defined on the Demultiplexing (NextSeq 1000/2000 Sequencing v2.1) step. The fields are used to display the demultiplexing result metrics for individual library in the sample pool.

The following table lists the global fields that are configured on the Demultiplexing (NextSeq 1000/2000 Sequencing v2.1) step.

Table 340 Global Field Configuration for Demultiplexing (NextSeq 1000/2000 Sequencing v2.1) Step

Field Name	Field Type	Field Constraints/Options
# of >= Q30 Bases (PF)	Numeric	• Required: No • Read Only: Yes
# One Mismatch Index Reads	Numeric	• Required: No • Read Only: Yes
# Perfect Index Reads	Numeric	• Required: No • Read Only: Yes
# Reads	Numeric	• Required: No • Read Only: Yes
Mean Quality Score (PF)	Numeric	• Required: No • Read Only: Yes

User Interaction

Refer to *NextSeq 1000/2000 Integration v2.2 Validation and Troubleshooting* on page 1651 for details.

How the NextSeq 1000/2000 Integration Works

Sample Sheet Generation and Planned Run Creation

On the Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.1) step, when the Validate Run Setup and Create Planned Run automation is triggered, all the run and analysis analysis parameters entered in the Run Details screen is sent to Illumina Connected Analytics. ICA validates the run and analysis configuration, and sends an error message to Clarity LIMS if the validation fails. If the validation passes, ICA generates the sample sheet (and sends back to Clarity LIMS) and/or creates a planned run depending on the Run Mode selected.

- Local run mode: Only the sample sheet is generated and stored on Clarity LIMS. The sample sheet contains all the run and analysis configuration required to start the run on the instrument.
- Hybrid/Cloud run mode: A planned run is created on ICA. This planned run contains all the run and analysis configuration required to start the run on the instrument. The sample sheet is also generated and stored on Clarity LIMS for reference purpose.

Refer to *Step-by-Step Instruction to Start A Sequencing Run On Instrument With Different Run Modes* on page 1648 for details on how to start a run using different run mode.

Run Status, Primary Metrics and Analysis Results Parsing and Recording

After the sequencing run is started on the instrument, NextSeq 1000/2000 Control Software notifies BaseSpace Sequence Hub that the sequencing run has started.

BSSH then does the following actions:

- Local run mode: Creates a new run on the BaseSpace Sequence Hub database with the run configuration information received from NextSeq 1000/2000 Control Software.
- Hybrid/Cloud run mode: Creates a new run on the BaseSpace Sequence Hub database by copying the run configuration information from the planned run created in ICA, using a special Cloud Run ID received from NextSeq 1000/2000 Control Software.

After the run is created on BaseSpace Sequence Hub, a run update event message that carries the RunStarted equivalent message is created on BSSH and delivered through ICA and, eventually, to Clarity LIMS SQS queue service. The message is grabbed and processed by SIS integration service.

Based on the reagent cartridge barcode information in the event message (see the following example), SIS looks in the queue of AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.1) step for a container with the same reagent cartridge barcode. When identified, the step is started automatically and the Run Status field on Record Details screen is updated to RunStarted. SIS then updates all the other step fields (eg, Current Read, Output Folder etc.) by parsing the details from the `RunParameters.xml` uploaded to BaseSpace Sequence Hub by NextSeq 1000/2000 Control Software.

```
#Example of an run update event
{
   "acl": [
      "wid:61dc14e8-d823-3bd1-bea3-66f9c8388254"
```

```
    ],
    "apiUrl":
    "https://api.basespace.illumina.com/v2/runs/r.ZvTfDXsNhUyh2kA5S4Zewg",
    "dateModified": "2020-03-26T09:19:58.5546497Z",
    "flowcellBarcode": "11M11COMC",
    "gdsFolderPath": "/Runs/200326_MP2-15_87_11M11COMC_r.ZvTfDXsNhUyh2kA5S4Zewg",
    "gdsVolumeName": "bssh.61dc14e8d8233bd1bea366f9c8388254",
    "id": "r.ZvTfDXsNhUyh2kA5S4Zewg",
    "instrumentRunId": "200326_MP2-15_87_11M11COMC",
    "name": "200326_MP2-15_87_11M11COMC",
    "reagentBarcode": "EC0001990-EC5",
    "sampleSheetName": "SampleSheet.csv",
    "status": "Running",
    "v1pre3Id": "194424236"
}
```


How the SIS Integration Service Handles Run Status

The NextSeq 1000/2000 Control Software continues to notify BSSH of any run status change throughout the course of the sequencing run. The run update event delivery process continues as described in the previous section.

The following table lists the run status details and how each status is handled by SIS integration service.

Run Status	Shown in Step	Details

RunStarted	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.1)	• Run Status field is updated to RunStarted.
		• All other step fields are updated based on RunParameters.xml detail obtained from BaseSpace Sequence Hub.
		• Reagent cartridge lot information received from BaseSpace Sequence Hub is compared against the details of the preaccessioned reagent cartridge on Clarity LIMS.
		  ◦ If the expiration date is different, it is updated to the date received from BaseSpace Sequence Hub.
		  ◦ If reagent cartridge is not found, it is created and set to Active.
		  ◦ If reagent cartridge is not in Active status, it is set to Active.
		• Multiline Sequencing Log field is updated.

Run Status	Shown in Step	Details
RunCompletedSuccessfully	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.1)	• Run Status field is updated to RunCompletedSuccessfully • Current Read and Current Cycle fields are updated to the final cycle number and read number based on `RunParameters.xml` detail obtained from BaseSpace Sequence Hub. • Run End Time field is updated based on RunParameters.xml detail obtained from BaseSpace Sequence Hub. • Sequencing run result (primary metrics) is downloaded from BaseSpace Sequence Hub and the fields in Sample Details table (eg, % Error Rate R1, % Phasing, etc.) are updated with the values. • Multiline Sequencing Log field is updated. • SIS autocompletes the step and routes the samples in the reagent cartridge container to the next step (ie, Demultiplexing (NextSeq 1000/2000 Sequencing v2.1) step if GenerateFastQ is selected as the Analysis Workflow in Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.1) step). Otherwise, the step is completed automatically and the samples are removed from the workflow.

Run Status	Shown in Step	Details
RunErroredOut	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.1)	• Run Status field is updated to RunErroredOut. • Current Read and Current Cycle fields are updated to the final cycle number and read number that the run stopped based on RunParameters.xml detail obtained from BaseSpace Sequence Hub. • Run End Time field is updated based on RunParameters.xml detail obtained from BaseSpace Sequence Hub. • Sequencing run result (primary metrics) is downloaded from BaseSpace Sequence Hub and the fields in Sample Details table (eg, % Error Rate R1, % Phasing, etc.) are updated with the values. • Multiline Sequencing Log field is updated. • SIS does not autocomplete the step. Complete the step manually.

Run Status	Shown in Step	Details
RunAbortedByUser	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.1)	• Run Status field is updated to RunAbortedByUser. • Current Read and Current Cycle fields are updated to the final cycle number and read number that was aborted based on RunParameters.xml details obtained from BaseSpace Sequence Hub. • Run End Time field is updated based on RunParameters.xml detail obtained from BaseSpace Sequence Hub. • Sequencing run result (primary metrics) is downloaded from BaseSpace Sequence Hub and the fields in Sample Details table (eg, % Error Rate R1, % Phasing, etc.) are updated with the values. • Multiline Sequencing Log field is updated. • SIS does not autocomplete the step. Complete the step manually.
AnalysisStarted	Demultiplexing (NextSeq 1000/2000 Sequencing v2.1)	• SIS starts the step automatically for the reagent cartridge container. • Multiline Sequencing Log field is updated.

Run Status	Shown in Step	Details
AnalysisCompleted	Demultiplexing (NextSeq 1000/2000 Sequencing v2.1)	• SIS downloads the `Demultiplex_stats.csv` file, which contains the demultiplexing result from BaseSpace Sequence Hub. • The file is parsed and details are recorded in the Sample Details table for each library in the library pool. • Hidden master step fields are updated with information from BaseSpace Sequence Hub. In particular, Analysis Status is updated to "Complete". • Multiline Sequencing Log field is updated. • SIS does not autocomplete the step. Assign the QC flag to the individual library and manually complete the step.
AnalysisFailed	Demultiplexing (NextSeq 1000/2000 Sequencing v2.1)	• Hidden step fields are updated with information from BSSH. In particular, Analysis Status is updated to either "TimedOut" or "Aborted". • Multiline Sequencing Log field is updated. • SIS does not auto-complete the step. Assign the QC flag to the individual library and manually complete the step.

Step-by-Step Instruction to Start A Sequencing Run On Instrument With Different Run Modes

Local Run Mode

1. On the NextSeq 1000/2000 Control Software interface, select the menu button and select Settings.
2. Select Local Run Setup and Proactive, Run Monitoring and Storage*.
3. [Optional] Enter the default output folder location.
4. Select Save.
5. On the main screen, select Start.

6. Sign in to the BaseSpace Sequence Hub (BSSH) account that was used during NextSeq 1000/2000 Integration package installation setup.
   a. Select the workgroup (if applicable).
   b. Select Next.
7. Select Choose, and a file browser pops up.
8. Browse and select the sample sheet generated during the Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.0) step.
   When selected, the sample sheet has its content validated. After the validation passes, run and analysis configuration is parsed and autopopulated in the next page.
9. Select Review to review the sequencing run settings.
10. Select Load and manually load the reagent cartridge and flow cell.
11. Select Sequence and the pre-run check proceeds.
    After the pre-run check passes, the sequencing run starts automatically.

Hybrid or Cloud Run Mode

1. On the NextSeq 1000/2000 Control Software interface, select the menu button and select Settings.
2. Select Online Run Setup and Proactive, Run Monitoring and Storage*.
3. [Optional] Enter the default output folder location.
4. Select Save.
5. On the main screen, select Start.
6. Sign into the BaseSpace Sequence Hub (BSSH) account that was used during NextSeq 1000/2000 Integration package installation setup.
   a. Select the workgroup (if applicable).
   b. Select Next.
   Planned runs that were created in the Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.0) step, using Hybrid or Cloud run mode, display as options.
7. Select the run and select Review to review the sequencing run settings.
8. Select Load and manually load the reagent cartridge and flow cell.
9. Select Sequence and the pre-run check proceeds.
   After the pre-run check passes, the sequencing run starts automatically.

Components Installed

The following sections describe the various components that are installed by default as part of this integration. These components include files, properties, reagent categories/label groups, reagent kits, control types, and containers. Information on installed workflows, protocols, steps, and automation points is provided in the *Workflows, Protocols, and Steps* on page 1672 section.

Reagent Categories/Label Groups

- TruSeq HT Adapters v2 (D7-D5)

Reagent Kits

- Resuspension Buffer (RSB)
- NextSeq 1000/2000 Reagent Cartridge

Container Types

- Tube
- NextSeq 1000/2000 Reagent Cartridge

Control Types

- PhiX v3

This integration supports:

- NextSeq 1000/2000 Reagent Cartridge with barcode provided in the format [A-Za-z]{2}[0-9]{7}-[A-Za-z0-9]{4}
  - Example: EC1234567-EC03

Rules and Constraints

- The workflow configuration contains several validation checks. To make sure that the calculations work properly, it is important that you do not disable any of this validation logic. The validation checks determine:
  - Which samples, and how many, can enter each step together.
  - Which samples, and how many, can be pooled together.
- The NextSeq 1000/2000 reagent cartridge barcode must be unique. There should not be multiple NextSeq 1000/2000 reagent cartridge containers in the system with the same name.
- Reagent labels (indexes) must be unique.
- One library pool can only contain one library or control with no label (index).
- Do not manually start or complete the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.1) step. This step is a fully automated step, and the SIS integration service does not update samples correctly if they have been manually started.
- Do not manually start the Demultiplexing (NextSeq 1000/2000 Sequencing v2.1) step. This step is semiautomated, and the SIS integration service does not update the demultiplexing result correctly if they have been manually started.

- For the automated run to start successfully, select Validate Run Setup and Create Planned Run in the Load to Reagent Cartridge step.

NextSeq 1000/2000 Integration v2.2 Validation and Troubleshooting

This section explains how to validate the installation of the Illumina NextSeq 1000/2000 Integration Package v2.2.

The validation process involves:

- Running samples through the Library Prep Validation v2.0.4 workflow.
  - The workflow contains a single-step protocol that models the library prep workflow required to produce libraries tagged with index sequences. At the end of the step, these libraries are routed to NextSeq 1000/2000 Sequencing v2.1 workflow.
- Running the libraries through the NextSeq 1000/2000 Sequencing v2.1 workflow. This process validates the following information:
  - Successful sequential step advancement of samples in the following steps:
    1. Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.1)
    2. Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.1)
    3. AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.1)
    4. [Optional] Demultiplexing (NextSeq 1000/2000 Sequencing v2.1)
  - Automatic validation of run setup information before sample sheet generation or planned run creation.
  - Automated generation of a sample sheet. The sample sheet is used to start the sequencing run on NextSeq 1000/2000 Control Software via Local run mode.
  - Automated creation of a planned run on Illumina Connected Analytics (ICA). The NextSeq 1000/2000 Control Software retrieves the planned run to start the run via Hybrid or Cloud run mode.
  - Automated tracking of the NextSeq 1000/2000 sequencing run and parsing of run statistics from BaseSpace Sequence Hub (BSSH) into Clarity LIMS using SIS Integration Service.
  - Automated tracking of the secondary analysis using DRAGEN (up to BCL Convert only).
  - Parsing of demultiplexing metrics from BaseSpace Sequence Hub (BSSH) into Clarity LIMS using SIS integration service.

Before running the validation steps below, these steps assume that the NextSeq 1000/2000 Sequencing v2.2 Integration Package is installed and you have imported the default Clarity LIMS configuration.

For information on how the integration works, refer to *NextSeq 1000/2000 Integration v2.2 Configuration and User Interaction* on page 1617.

Activate Workflow, Create Project, Add and Assign Samples

The following steps set up Clarity LIMS in preparation for running samples through the Library Prep Validation v2.0.4 and NextSeq 1000/2000 Sequencing v2.1 workflows.

1. In the Configuration tab of Clarity LIMS under Workflows, activate both the Library Prep Validation v2.0.4 and NextSeq 1000/2000 Sequencing v2.1 workflows.
2. On the Projects and Samples screen, create a project and add samples to it.

   Use only alphanumeric, dash, and underscore characters in the submitted sample names. Failing to do so causes sample sheet validation failure in Load To Reagent Cartridge step.

3. Assign the samples to the Library Prep Validation v2.0.4 workflow.

Library Prep Protocol: Library Prep Validation v2.0.4

This single-step protocol models the library prep required to produce libraries tagged with index sequences that are ready for the NextSeq 1000/2000 Sequencing v2.1 workflow.

Run Library Prep Validation v2.0.4 Step

1. In Lab View, locate the Library Prep Validation v2.0.4 protocol. The samples are queued for the Library Prep Validation v2.0.4 step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select the label groups to use.
   - TruSeq HT Adapters v2 (D7-D5) is the default label group delivered by Library Prep Validation v2.0.4 protocol.
   - For other reagent labels (indexes), manually create them in the Configuration screen under Consumables → Labels.

   For more information on how to create reagent label group, refer to the *Add and Configure Labels and Label Groups* section in the Clarity LIMS Core Functionality documentation.

4. Select Begin Work.
5. On the Placement screen:
   a. In the Placed Samples area in the Container field, scan or enter the barcode of the destination container.
   b. Select the samples from the Samples to be Placed area, then drag them over to the container wells on the right.
   c. Select Add Labels.
6. On the Add Labels screen, the Labels to Add list is populated with labels from the TruSeq HT Adapters v2 (D7-D5) label group. This label group is the only one configured in the out-of-the-box integration. It is used by default in this step.
   a. Select labels on the left, and drag them over to the container on the right to assign them to placed samples.

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b. Select Record Details.

7. On the Record Details screen:
   a. In the Sample Details table, enter values for Molarity (nM) for all the individual sample. Enter values individually or use the copy/paste functionality to enter multiple values from a spreadsheet.
      - Molarity (nM) values are used in Calculate Volume automation script in Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.1) step.

      If Molarity (nM) values are not entered, the Calculate Volume automation script does not work properly.

   b. The Sequencing Instrument column indicates the instrument to be used for the run. Select NextSeq 1000/2000.
   c. Populate the fields in this column using one of the following methods:
      - To populate fields individually for each sample, select from the drop-down list in each row.
      - To populate fields all at one time, use the Copy and Paste buttons to enter multiple values from a spreadsheet (see the following note).

   Alternatively, select inside the table and press Ctrl + A to select all rows. At the top of the table, select Sequencing Instrument, then select NextSeq 1000/2000 from the adjacent drop-down list. Select Apply.

      If you are working with many samples, you can use the copy/paste functionality to enter multiple values from a spreadsheet.

      a. Select the Copy button to copy the sample information from Clarity LIMS to the clipboard.
      b. Open a blank Excel document and paste the contents of the clipboard into the spreadsheet.
      c. In the spreadsheet, enter the Molarity (nM) and Sequencing Instrument values. Select the entire spreadsheet, including the headers, (Ctrl + A) and copy (Ctrl + C) the information to the clipboard.
      d. In Clarity LIMS, select the Paste button. Paste the contents of the clipboard (Ctrl + V) into the Update Table window and then select Update to paste into the Sample Details table.

8. Select Next Steps.
   This selection triggers the Next Steps automation, which sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step successfully.
9. On the Assign Next Steps screen in the Samples table, the Next Step for all samples is prepopulated with Remove from workflow.

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> Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.

10. Select Finish Step.
    On exit from the step, the Routing Script automation is triggered. This automation assigns samples to the first step of the NextSeq 1000/2000 Sequencing v2.1 workflow, which is Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.1) step.

NextSeq 1000/2000 Sequencing v2.2 Protocol

Step 1: Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.1)

1. In Lab View, locate the NextSeq 1000/2000 Sequencing v2.1 protocol. The samples are queued for the Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.1) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Pooling screen:
   a. Create a pool by dragging samples into the Pool Creator.

   > Do not create more than one pool for this step. The Calculate Volumes automation in this step supports one pool only.

   b. Type a name for the pool or accept the default name (Pool #1).
   c. Select Record Details.
5. On the Record Details screen, the Reagent Lot Tracking section tracks the Resuspension Buffer (RSB) lot information used in the step.
   To add and activate additional reagent lots, perform the following steps:
   a. In a new browser tab, open an additional instance of Clarity LIMS.
   b. Navigate to the Consumables configuration screen.
   c. Select the RSB reagent kit and select New Lot.
   d. Enter the lot details and select Save.
   e. For Status of Reagent Lot, select Active.
6. In the Reagent Lot Tracking section, select from the active lots displayed in the drop-down list.
7. The Step Details area contains two required fields:
   - Final Loading Concentration (pM)—The value entered in this field is the recommended final loading concentration specified in the *Illumina NextSeq 1000/2000 Sequencing System Guide*. The value is dependent on the library type. The default drop-down list contains the values 650, 750, 1000, and 2000. A custom value is acceptable.

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- Final Loading Volume (µl)—The value in this field is the final loading volume of the pool into the reagent cartridge. The field is prepopulated with the configured default value, 24 µl, specfied in the *Illumina NextSeq 1000/2000 Sequencing System Guide*. The value is editable when more volume is necessary.

8. Select Calculate Volumes. This selection triggers the Calculate Volumes automation. This automation calculates the volume required for each library to form a pool that has the concentration and volume specified in the step details fields.
   The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains volume information of each of the samples and RSB buffer to add to the pool. Select the file to download it, then open it in Excel.
9. In the Sample Details table, select the pool icon to view details on the pool composition.
10. Select Next Steps. This selection triggers the Set Next Step automation, which sets the next step for samples to ADVANCE, advancing them to the next step in the protocol. The next step is Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.1).
    On the Assign Next Steps screen, the next step for samples is already set to the next step in the workflow. The next step is Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.1).
11. Select Finish Step.

At the end of this step, the pool of samples automatically advances to Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.1) step.

Step 2: Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.1)

1. In Lab View, locate the NextSeq 1000/2000 Sequencing v2.1 protocol. The pool of samples is queued for the Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.1) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
   The Validate Single Input automation is triggered. This automation checks that there is only one container input to the step.
4. On the Placement screen:
   a. Drag the pool into the NextSeq 1000/2000 Reagent Cartridge in the Placed Samples area.
   b. Scan or type the barcode of the reagent cartridge into the NextSeq 1000/2000 Reagent Cartridge field.
   c. Select Record Details.
   On exit of the Placement screen, the Validate Reagent Cartridge Barcode automation checks that the reagent cartridge barcode conforms to the barcode mask [A-Za-z]{2}[0–9]{7}-[A-Za-z0-9]{4}. If not, an error message displays.
   On entry to the Record Details screen, the Retrieve Analysis Workflow Versions automation fetches the available analysis workflow versions from ICA. It then updates the preset values of both Local Analysis Workflow Versions & Cloud Analysis Workflow Versions field.

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5. On the Record Details screen, the Reagent LotTracking section tracks the NextSeq 1000/2000 Reagent Cartridge lot information used in the step. Follow the steps in Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.1) step if you need to add a new lot.
6. In the Reagent Lot Tracking section, select from the active lots displayed in the drop-down list.
7. The fields displayed on the Record Details screen are used to create planned run and generate the sample sheet file.
   - Run Name—Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.
   - Instrument Type—Select from preset options: NextSeq 1000, NextSeq 2000.
   - Run Mode—Select from preset options: Local, Hybrid, Cloud. See *Run Mode Details* on page 1657 for more information on different run mode.
   - Paired End—Select from preset options: True or False.
   - Read 1 Cycles—Select from preset options (151, 101, 51) or type a custom value.
   - Read 2 Cycles—Select from preset options (151, 101, 51) or type a custom value.
   - Index Reads—Select from preset options: No Index, Single Index, Dual Index.
   - Index Read 1—Select from preset options (0, 6, 8) or type a custom value.
   - Index Read 2—Select from preset options (0, 6, 8) or type a custom value.
   - Analysis Workflow—Select from preset options: GenerateFASTQ or None. See *Analysis Workflow Details* on page 1658 for more information.
   - Local Analysis Workflow Versions—[Optional] (default= None). Only mandatory when Run Mode = Local/Hybrid and Analysis Workflow = GenerateFASTQ. Select according to the DRAGEN version installed on the instrument.
   - Cloud Analysis Workflow Versions—[Optional] (default= None). Only mandatory when Run Mode = Cloud and Analysis Workflow = GenerateFASTQ. Select the cloud DRAGEN version to be used.
   - FASTQ Compression Format:
     - If Run Mode = Local/Hybrid, Analysis Workflow = GenerateFASTQ, and Local Analysis Workflow Version >= 3.7.4, select gzip or DRAGEN.
     - If Run Mode = Cloud, Analysis Workflow = GenerateFASTQ, and Local Analysis Workflow Version >= 3.7.4, field is set to gzip automatically.
     - Otherwise, select None.
   - Adapter Sequence Read 1—[Optional] Enter the Read 1 adapter sequence of the index adapter kit.
   - Adapter Sequence Read 2—[Optional] Enter the Read 2 adapter sequence of the index adapter kit.

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- Barcode Mismatches Index 1—[Optional] Select from preset options: 0, 1, 2. Leave it blank if you are unsure.
- Barcode Mismatches Index 2—[Optional] Select from preset options: 0, 1, 2. Leave it blank if you are unsure.
- Override Cycles—[Optional] String used to specify UMI cycles and mask out cycles of a read, eg, N1Y150;I8;I7N1;Y141U10. Leave it blank if you are unsure.
- Instructions — Read-only

8. On the Record Details screen, select Validate Run Setup and Create Planned Run. This selection triggers the automation script, which does the following actions:
   - Validates the parameters entered on the Record Details screen.
   - Create the planned run and send it to Illumina Connected Analytics (ICA) if Run Mode is Hybrid or Cloud.
   - Generates the sample sheet and attaches it to the placeholder in the Files area of the Record Details screen for all Run Mode types.
9. Select Next Steps.
   On the Assign Next Steps screen, the next step for samples is set to the next step in the workflow. The next step is AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.1).
10. Select Finish Step.

At the end of this step, the pool of samples automatically advances to and is queued for the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.1) step. The sequencing run is ready to be started. For details on how to start the sequencing run for different run modes, refer to *NextSeq 1000/2000 Integration v2.2 Configuration and User Interaction* on page 1617.

Run Mode Details

The run mode applies to all runs and determines how the run and analysis parameters are configured and how to analyze data.

- Local Mode—NextSeq 1000/2000 Integration service generates the sample sheet. Run/Analysis configuration is imported manually into NextSeq 1000/2000 instrument via a sample sheet to start the run. The downstream secondary analysis is done using the onboard DRAGEN module.
- Hybrid Mode—NextSeq 1000/2000 Integration service sends the run/analysis configuration to Illumina Connected Analytics. Run/Analysis configuration is downloaded to NextSeq 1000/2000 instrument via ICA to start the run. The downstream secondary analysis is done using the onboard DRAGEN module.
- Cloud Mode—NextSeq 1000/2000 Integration service sends the run/analysis configuration to ICA. Run/Analysis configuration is downloaded to NextSeq 1000/2000 instrument via ICA to start the run. The downstream secondary analysis is performed in the cloud.

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Analysis Workflow Details

The analysis workflow selection determines if the sample is:

- Advanced to the Demultiplexing (NextSeq 1000/2000 Sequencing v2.1) step
- Completed at the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.1) step

- GenerateFASTQ—After the sequencing run completes, NextSeq 1000/2000 integration service does not immediately complete the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.1) step. Instead, only after the secondary analysis completes (or errors out) does the integration service automatically complete the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.1) step. Samples are then advanced to the Demultiplexing (NextSeq 1000/2000 Sequencing v2.1) step.
- None—None is selected to skip Demultiplexing (NextSeq 1000/2000 Sequencing v2.1) step. NextSeq 1000/2000 integration service automatically completes the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.1) and removes the samples from the workflow.

Step 3: AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.1)

This step is fully automated.

Do not add samples to the Ice Bucket or start or complete the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.1) step. The integration does this action automatically.

The integration starts the step automatically and data from the run is parsed back to Clarity LIMS. No user interaction is required. However, you can open and review the various stages of the step in Clarity LIMS. Do not perform any action when reviewing the data.

Read summary metrics are recorded for the library pool in the Step Details section and the Sample Details table.

Values are populated in the following step UDFs/master step fields:

Run Name	Run Status	Output Folder
Current Read	Current Cycle	Secondary Analysis Workflow
Flow Cell ID	Flow Cell Lot Number	Reagent Cartridge ID
Reagent Cartridge Lot Number	Instrument Platform	Instrument Type
Instrument ID	Instrument Control Software Version	RTA Version
Sequencing Log		

The summary metrics (per run level) populate in the following UDFs/global fields.

- % Bases >=Q30 R1

- % Bases >=Q30 R2
- % Error Rate R1
- % Error Rate R2
- Yield (Gb) R1
- Yield (Gb) R2
- Reads PF R1
- Reads PF R2
- %PF R1
- %PF R2
- % Aligned R1
- % Aligned R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- Cluster Density R1
- Cluster Density R2

At the end of this step, the pool of samples undergoes either one of the following two actions:

1. If the Analysis Workflow field in Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.1) step is set to GenerateFASTQ, the pool automatically advances to and is queued for the Demultiplexing (NextSeq 1000/2000 Sequencing v2.1) step.
2. If the Analysis Workflow field in Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.1) step is set to None, the pool automatically completes AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.1) step and gets removed from the workflow.

Step 4: Demultiplexing (NextSeq 1000/2000 Sequencing v2.1)

This step is semi-automated.

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For the demultiplexing information in the Sample Details table to display correctly, the following conditions must be met:

- The DRAGEN version must be earlier than v3.9.3.
- The samples in the run must not repeat.
- The run must be for the single lane flow cell (P1 or P2).

Do not add samples to the Ice Bucket or start or complete the step. The integration completes these actions automatically.

The integration starts the step automatically and demultiplexing data from the GenerateFASTQ secondary analysis is parsed back to Clarity LIMS. Review the data parsed and assign QC, depending on the criteria set, and complete the step.

In the Record Details screen:

1. Review demultiplexing data.
   - Demultiplexing metrics are recorded for the library pool in the Sample Details table. Metrics include columns for # Reads, # Perfect Index Reads, # One Mismatch Index Reads, and # of >= Q30 Bases (PF).
   - The `Demultiplex_stats.csv` file generated by the Dragen onboard or Cloud analysis module is stored under the Files section.
2. Assign QC flags to all the individual sample. There are two ways of doing this step.
   a. Manually assign QC flags through the QC column in Sample Details table.
   b. Automatically assign QC flag by running Assign QC flags automation. This option is for scenarios where a huge number of libraries are involved.

   In the Step Details section, the following step fields are visible. (N is the number of criteria. You can use one or more criteria.)
   - Criteria N - Source Data Field—Select from preset options eg # Reads.
   - Criteria N - Operator—Select from preset options, for example, >= (greater than or equal to).
   - Criteria N - Threshold Value—Enter the desired threshold value.
3. After filling up the criteria fields, select Assign QC flags. This selection triggers the automation script, which loops through each library in the pool and apply QC flag base on the criteria set previously.
   This automation also generates an AssignQC Result file under Files section.
4. Select Next Steps.
   On the Assign Next Steps screen, the Next Step field for all samples is prepopulated with Mark protocol as complete.
5. Select Finish Step.

At this point, the whole NextSeq 1000/2000 Integration workflow is fully validated.

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Troubleshooting

If an automation trigger does not appear to run its corresponding scripts, see *Troubleshooting Automation* in the *Clarity LIMS API and Database* documentation.

If the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.1) step starts but does not finish, complete the following steps:

1. Log in to Clarity LIMS using the default user account and use one of the following methods to open the step in Clarity LIMS:
   - Method 1: In Lab View, find the step in the Recent Activities pane.
   - Method 2: Search for the step in Clarity LIMS using reagent cartridge barcode as the search term.
2. On the Record Details screen, locate the Sequencing Log field.
   The multiline text field contains logging information.

If you cannot reach the Record Details screen, or if the Sequencing Log field does not contain enough information to resolve the issue, contact the Clarity LIMS support team. Supply the relevant information from the troubleshooting steps already performed.

NextSeq 1000/2000 Integration v2.2 Manual Upgrade

NextSeq 1000/2000 Integration v2.2 supports future ICA services and includes new custom fields and modifications to existing automations. The integration package requires the NextSeq 1000/2000 Sequencing (v2.0 or v2.1) workflow. You can install this workflow through the Illumina Preset Protocols (IPP). If you do not want to upgrade through the IPP, you can manually upgrade the workflow configuration.

The upgrade steps differ, depending on the integration version.

- If upgrading from NextSeq 1000/2000 Integration v1.x to v2.2, follow the instructions in all three workflow upgrade sections.
- If upgrading from v2.0 to v2.2, follow the instructions in *Upgrade the NextSeq1000/2000 Workflow (v2.0 to v2.1)* and *Upgrade the NextSeq1000/2000 Workflow (v2.1 to v2.2)*.
- If upgrading from v2.1 to v2.2, follow the instructions in *Upgrade the NextSeq1000/2000 Workflow (v2.1 to v2.2)*.

Upgrade the NextSeq1000/2000 Workflow (v1.x to v2.0)

To upgrade from NextSeq1000/2000 Sequencing v1.x workflow to v2.0, update the master step sample file placeholder and modify the automation.

Update File Placeholder

Update the master step sample file placeholder as follows.

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1. In Clarity LIMS, under Configuration, select the Lab Work tab.
2. In the Master Steps list, select the Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.0) master step.
3. Select the Record Details milestone.
4. In the Step File Placeholders section, change File Name from Log File2 to Sample Sheet Generation Log File.
5. Select Save.

Modify Existing Automation

Modify the existing automation as follows.

1. From Configuration, select the Automation tab.
2. Under the Step Automation tab, select the Validate Run Setup and Create Planned Run automation.
3. In the command line, remove `/v1` from the path to the `nextseq1k2k-automation.jar` file. After this change, the path will be as follows.

```
/opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/nextseq1k2k-automation.jar.
```

4. Select Save.

Upgrade the NextSeq1000/2000 Workflow (v2.0 to v2.1)

To upgrade from NextSeq1000/2000 Sequencing v2.0 workflow to v2.1, complete the following steps:

- Create custom fields.
- Create Retrieve Analysis Workflow Versions automation.
- Update the existing automation.

Create Custom Fields

1. From Configuration, select the Custom Fields tab.
2. Under the Master Step Fields tab, select the Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing) master step.
3. Create custom fields using the following table.

Table 341 Custom Fields for Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing) Master Step

Name	Field Type	Field Options	Additional Options
Instructions	Multiline Text	• Required—No • Read Only—Yes	Default—If GenerateFASTQ analysis is required, please select the DRAGEN version in Cloud Analysis Workflow Versions (for Cloud run mode) or Local Analysis Workflow Versions (for Local/Hybrid run mode) custom fields.
Local Analysis Workflow Versions	Text Dropdown	• Required—No • Read Only—No • Custom Entries—No	For None, select Yes under Set as Default?
Cloud Analysis Workflow Versions	Text Dropdown	• Required—No • Read Only—No • Custom Entries—No	For None, select Yes under Set as Default?
FASTQ Compression Format	Text Dropdown	• Required—Yes • Read Only—No • Custom Entries—No	• For gzip, select Yes under Set as Default? • DRAGEN • None

4. Select Save.

Create Retrieve Analysis Workflow Versions Automation

1. From Configuration, select the Automation tab.
2. Under Automation Details, select New Automation.
3. Create an automation named Retrieve Analysis Workflow Versions.
4. Update the command line as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/nextseq1k2k-
automation.jar script:retrieve_analysis_workflow_versions -i {stepURI:v2} -u
{username} -p {password} -l {compoundOutputFileLuid2}"
```

5. Under Automation Use, enable this automation on the Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing) master step.
6. From Configuration, select Lab Work.

7. In the Master Steps list, select the Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing) master step.
8. Set the options as follows.
   - Trigger Location—Record Details
   - Trigger Style—Automatic upon entry

Modify Existing Automation

1. From Configuration, select the Automation tab.
2. Under the Step Automation tab, select the Validate Run Setup and Create Planned Run automation.
3. Update the command line as follows.
   Changes to the existing command line are in red.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-exp 'if (!step.::Run Name::.matches(::[a-zA-Z0-9-_.]+::)) { fail(::Run Name contains prohibited characters. Allowed characters are: a-z, A-Z, 0-9, -, _, and .::) }; if (step.::Run Name::.length() > 255) { fail(::Run Name shall not exceed 255 characters.::) }; if (step.::Index Reads:: == ::No Index::) { if (step.::Index Read 1:: != 0 || step.::Index Read 2:: != 0) { fail(::Index Read 1 and Index Read 2 must be 0 if the Index Reads is No Index.::) }} else { if (step.::Index Reads:: == ::Single Index::) { if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) { fail(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if the Index Reads is Single Index.::) }} else { if (step.::Index Read 1:: == 0 || step.::Index Read 2:: == 0) { fail(::Index Read 1 and Index Read 2 must be greater than 0 if the Index Reads is Dual Index.::) }}}; if (step.::Paired End::.toBoolean()) { if (step.::Read 2 Cycles:: == 0) { fail(::Read 2 Cycles must not be zero if it is Paired End read.::) }} else { if (step.::Read 2 Cycles:: != 0 || step.::Index Read 2:: != 0) { fail(::Read 2 Cycles and Index 2 Cycles must be 0 if it is not Paired End Read.::) }}; if (step.hasValue(::Adapter Sequence Read 1::) && !step.::Adapter Sequence Read 1::.matches(::[ACTG+]+::)) { fail(::Adapter Sequence Read 1 contains prohibited characters. Allowed characters are: ACTG+::) }; if (step.hasValue(::Adapter Sequence Read 2::) && !step.::Adapter Sequence Read 2::.matches(::[ACTG+]+::)) { fail(::Adapter Sequence Read 2 contains prohibited characters. Allowed characters are: ACTG+::) }; if (step.::Analysis Workflow:: == ::None::) { step.::Cloud Analysis Workflow Versions:: = ::None::; step.::Local Analysis Workflow Versions:: = ::None::; step.::FASTQ Compression Format:: = ::None::; }; if (step.::Analysis Workflow:: == ::GenerateFASTQ:: && step.::Run Mode:: == ::Cloud::) { step.::Local Analysis Workflow Versions:: = ::None::; if
```


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```
(step.::Cloud Analysis Workflow Versions:: == ::None::) { fail(::Invalid option selected for Cloud Analysis Workflow Versions field.::); }}; if (step.::Analysis Workflow:: == ::GenerateFASTQ:: && (step.::Run Mode:: == ::Local:: || step.::Run Mode:: == ::Hybrid::)) { step.::Cloud Analysis Workflow Versions:: = ::None::; if (step.::Local Analysis Workflow Versions:: == ::None::) { fail(::Invalid option selected for Local Analysis Workflow Versions field.::); }}; if (step.hasValue(::Override Cycles::) && !step.::Override Cycles::.matches(::[YNIU0-9;]+::)) { fail(::Override Cycles contains prohibited characters. Allowed characters are: Y, N, I, U, 0-9 and ;. Example: N1Y150;I8;I7N1;Y141U10.::) }' \
-log {compoundOutputFileLuid1} \
-t true \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/nextseq1k2k-automation.jar script:generate_v2_sample_sheet -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid2} -c {compoundOutputFileLuid0}"
```

4. Select Save.

Upgrade the NextSeq1000/2000 Workflow (v2.1 to v2.2)

To upgrade from NextSeq1000/2000 Sequencing v1.x workflow to v2.0, complete the following steps:

- Create custom fields.
- Modify the existing NextSeq 1000/2000 workflow.
- Add custom fields to steps.
- Update the step file placeholders.
- Modify the existing automation.

Create Custom Fields

1. In Clarity LIMS, under Configuration, select the Custom Fields tab.
2. Under the Global Fields tab, select Submitted Sample.
3. Create a custom field named Illumina Universal Sample Identifier.
4. For Field Type, select Text.
5. For Field Options, select the following:
    - Required Field—No
    - Read Only—No
6. Select Save.
7. Select Measurement.
8. Create a custom field named Mean Quality Score (PF).

9. For Field Type, select Numeric.
10. For Field Options, select the following:
    - Required Field—No
    - Read Only—Yes
11. Select Save.

Modify the Existing NextSeq 1000/2000 Workflow

Create custom fields for master steps in the workflow as follows.

1. From Configuration, select the Custom Fields tab.
2. Under the Master Step Fields tab, select the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing) master step.
3. Create custom fields using the following table.

Table 342 Custom Fields for AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing) Master Step

Name	Field Type	Field Options
BaseSpace Run ID	Text	• Required—No • Read Only—Yes
Flow Cell Mode	Text	• Required—No • Read Only—Yes
Instrument Run ID	Text	• Required—No • Read Only—Yes
Run Start Time	Text	• Required—No • Read Only—Yes
Run End Time	Text	• Required—No • Read Only—Yes
Secondary Analysis Mode	Text	• Required—No • Read Only—Yes
Secondary Analysis Platform Version	Text	• Required—No • Read Only—Yes
SkipObdd	Text	• Required—No • Read Only—Yes

4. Select Save.
5. Under the Master Step Fields tab, select the Demultiplexing (NextSeq 1000/2000 Sequencing) master step.
6. Create custom fields using the following table.

Table 343 Custom Fields for Demultiplexing (NextSeq 1000/2000 Sequencing) Master Step

Name	Field Type	Field Options
Analysis Completed Time	Text	• Required—No • Read Only—Yes
Analysis Started Time	Text	• Required—No • Read Only—Yes
Analysis Status	Text	• Required—No • Read Only—Yes
BSSH Analysis ID	Text	• Required—No • Read Only—Yes

7. Select Save.

Add Custom Fields to Steps

1. From Configuration, select the Custom Fields tab.
2. Under the Master Step Fields tab, select the Demultiplexing (NextSeq 1000/2000 Sequencing) master step.
3. Select the Ice Bucket milestone.
4. Under the Table Columns section, add Mean Quality Score (PF) from the Measurement drop-down to the list.

Update Step File Placeholders

1. From Configuration, select the Lab Work tab.
2. In the Master Steps list, select the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing) master step.
3. Select the Record Details milestone.
4. In the Step File Placeholders section, create a Log File.
5. Under Attachment, select Auto.
6. In the Master Steps list, select the Demultiplexing (NextSeq 1000/2000 Sequencing) master step.
7. Select the Record Details milestone.
8. In the Step File Placeholders section, change the second placeholder name to Log File.
9. In the Master Steps list, select the Library Pooling and Dilution (NextSeq 1000/2000 Sequencing) master step.
10. Select the Record Details milestone.
11. In the Step File Placeholders section , change the second placeholder name to Log File.

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Modify Existing Automations

Update the Set Next Step automation on the Demultiplexing (NextSeq 1000/2000 Sequencing v2.1) step as follows.

1. From Configuration, select the Automation tab.
2. Under the Step Automation tab, select the Set Next Step automation that is enabled on the Demultiplexing (NextSeq 1000/2000 Sequencing) step.
3. Change `compoundOutputFileLuid0` to `compoundOutputFileLuid1` in the existing command line as follows.
   Changes to the existing command line are in red.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -exp 'nextStep = ::ADVANCE::' -log {compoundOutputFileLuid1}"
```

4. Select Save.

Update the Retrieve Analysis Workflow Versions automation on the Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.1) step as follows.

1. From Configuration, select the Automation tab.
2. Under the Step Automation tab, select the Retrieve Analysis Workflow Versions automation that is enabled on the Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.1) step.
3. Change `compoundOutputFileLuid2` to `compoundOutputFileLuid1` in the existing command line as follows.
   Changes to the existing command line are in red.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/nextseq1k2k-automation.jar script:retrieve_analysis_workflow_versions -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1}"
```

4. Select Save.

NextSeq 1000/2000 Integration v2.2 Release Notes

These Release Notes describe the key changes to software components for the Clarity LIMS NextSeq 1000/2000 Integration Package since version 2.1.

This is an optional software update for customers interested in the features available with this release.

If upgrading from a version prior to v2.1, review the release notes for v2.1 for a list of features and bug fixes introduced in that version.

Compatibility

This version of the integration package is compatible with the following software:

- Clarity LIMS v5.3 and later

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- NextSeq 1000/2000 Instrument Control Software (NCS) v1.1 through 1.4.

New Features

Features provided with this release of the integration package are as follows:

- Support for Clarity LIMS v6.0.
- Fixes to address the following Apache log4j vulnerabilities:
  - CVE-2021-44228
  - CVE-2021-45046
  - CVE-2021-45105
  - CVE-2021-44832
- Tech refresh to upgrade third party libraries.
- Inclusion of non-PhiX control samples in v2 sample sheet.

Defect Repairs

None.

Known Issues

- A sequencing run error is triggered when instrument disk space is full. This error causes RunParameters.xml to be empty and the run event is not processed on Clarity LIMS.
- This error is unlikely to occur as pre-run checks on the instrument check for sufficient disk space before run is started.

Limitations

For the demultiplexing information in the Sample Details table to display correctly, the following conditions must be met:

- The DRAGEN version must be earlier than v3.9.3.
- The samples in the run must not repeat.
- The run must be for the single lane flow cell (P1 or P2).

NextSeq 1000/2000 Integration v2.1.1 Release Notes

The following release notes detail the key changes to software components for the Clarity LIMS NextSeq 1000/2000 Integration Package v2.1.1.

This update addresses Apache log4j vulnerabilities found in prior versions of the NextSeq 1000/2000 Integration Package.

Compatibility

This version of the integration package is compatible with the following software:

- Clarity LIMS v5.3 and later
- NextSeq 1000/2000 Instrument Control Software v1.1 and later (up to v1.4 only)

If you are currently using Clarity LIMS NextSeq1000/2000 Integration v.2.1.0, contact Illumina Support to update to v2.1.1.

Defect Repairs

This is a patch release to address the following Apache log4j vulnerabilities:

- CVE-2021-44228
- CVE-2021-45046
- CVE-2021-45105
- CVE-2021-44832

Known Issues

- A sequencing run error is triggered when instrument disk space is full. This error causes RunParameters.xml to be empty and the run event is not processed on Clarity LIMS.
- This error is unlikely to occur as pre-run checks on the instrument check for sufficient disk space before run is started.

Limitations

For the demultiplexing information in the Sample Details table to display correctly, the following conditions must be met:

- The DRAGEN version must be earlier than v3.9.3.
- The samples in the run must not repeat.
- The run must be for the single lane flow cell (P1 or P2).

NextSeq 1000/2000 Integration v2.1 Configuration and User Interaction

The Illumina NextSeq 1000/2000 Integration Package v2.1 supports the integration of Clarity LIMS to Illumina NextSeq 1000/2000 instruments.

This user and configuration guide describes the integration and includes information about preconfigured workflows, protocols, steps, and automations. This guide also lists installed components and provides configuration requirements, rules, and constraints.

For instructions on validating and troubleshooting the Illumina NextSeq 1000/2000 Integration, see *NextSeq 1000/2000 Integration v2.1 Validation and Troubleshooting* on page 1697.

The configuration provided in this integration has been established to support NextSeq 1000/2000 lab processes. Any configuration changes to protocols or workflows—including renaming protocols, steps, and fields—could break the process.

Overview

The integration includes the following features:

- Preconfigured NextSeq 1000/2000 Sequencing v2.0 workflow that maps to lab protocols and instrument runs.
- Preconfigured protocols:
  - Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.0)
  - Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.0)
  - AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.0)
  - Demultiplexing (NextSeq 1000/2000 Sequencing v2.0)
- Automatic validation of run setup information before sample sheet generation or planned run creation.
- Automated generation of a sample sheet. The sample sheet is used to start the sequencing run on NextSeq 1000/2000 Control Software via Local run mode.
- Automated creation of a planned run on Illumina Connected Analytics (ICA). The planned run created is retrieved by NextSeq 1000/2000 Control Software to start run via Hybrid or Cloud run mode.
- Automated tracking of the NextSeq 1000/2000 sequencing run and parsing of run statistics from BaseSpace Sequence Hub (BSSH) into Clarity LIMS using SIS integration service.
- Automated tracking of the secondary analysis using DRAGEN (up to BCL Convert only) and parsing of demultiplexing metrics from BaseSpace Sequencing Hub (BSSH) into Clarity LIMS using SIS integration service.
- [Optional] Preconfigured Library Prep Validation v2.0.3 workflow used for validation purposes only. The workflow contains a single-step protocol that models the library prep workflow required to produce libraries tagged with index sequences. At the end of the step, these libraries are routed to NextSeq 1000/2000 Sequencing v2.0 workflow. See *NextSeq 1000/2000 Integration v2.1 Validation and Troubleshooting* on page 1697.

Information on these features is provided in the following sections.

Prerequisites and Assumptions

It is assumed that samples entering the NextSeq 1000/2000 Sequencing v2.0 workflow have gone through library preparation and quantification processes. Before they are assigned to the workflow, samples have completed the following actions:

- Samples have been accessioned into Clarity LIMS.
- Samples have been run through QC and library prep.

For details on sample accessioning, see articles in the Samples section of the Clarity LIMS documentation for the appropriate version.

Samples can be assigned to the NextSeq 1000/2000 Sequencing v2.0 workflow automatically (using a routing script) or manually from the Projects and Samples dashboard. See the *Assign and Process Samples* section in the Clarity LIMS Core Functionality documentation.

Workflows, Protocols, and Steps

The Illumina NextSeq 1000/2000 Integration Package v2.1 includes two workflows:

- NextSeq 1000/2000 Sequencing v2.0
- Library Prep Validation v2.0.3 (optional, but recommended for validation purposes)

The following table lists and describes the protocols and steps included in these workflows.

Table 344 Illumina NextSeq 1000/2000 Integration Package v2.0 Protocols and Steps

Protocol Name	Protocol Purpose	Steps Included in Protocol
Library Prep Validation v2.0.3 Workflow		
Library Prep Validation v2.0.3	Included for validation purposes only, this protocol models the library prep steps required to advance samples to the NextSeq 1000/2000 Sequencing v2.0 workflow. The protocol contains a single step—Library Prep Validation v2.0.3. At the end of this step, a routing script sends the samples to the first step of the NextSeq 1000/2000 Sequencing v2.0 workflow. The first step is Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.0).	1. Library Prep Validation v2.0.3
NextSeq 1000/2000 Sequencing v2.0 workflow		

Protocol Name	Protocol Purpose	Steps Included in Protocol
NextSeq 1000/2000 Sequencing v2.0	This protocol models the lab processes of starting a NextSeq 1000/2000 sequencing run. Details of the steps: 1. Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.0) a. Samples are pooled and diluted to the final loading concentration with the help of the Calculate Volume script. 2. Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.0) a. The library pool from step 1 is then ready to be loaded to the NextSeq 1000/2000 reagent cartridge. b. Run and analysis information is validated. c. Sample sheet is generated and/or planned run is created. 3. AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.0) a. This step is a fully automated step that is started and completed automatically after the sequencing run is started and completed on the instrument side. b. All the sequencing run metadata (eg, run configuration, primary run metrics etc.) are recorded automatically. ⚠ Do not add samples to the Ice Bucket or start or complete the step. The integration does this action automatically. c. Samples complete the workflow at this step if GenerateFastQ secondary	1. Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.0) 2. Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.0) 3. AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.0) 4. Demultiplexing (NextSeq 1000/2000 Sequencing v2.0)

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Validation workflow

The Library Prep Validation v2.0.3 workflow allows for validation of the system after installation is complete. For details, see the *NextSeq 1000/2000 Integration v2.1 Validation and Troubleshooting* on page 1697.

Step Automations

Automation details are provided in the sections following the table.

For details on the validation workflow, see *NextSeq 1000/2000 Integration v2.1 Validation and Troubleshooting* on page 1697.

Table 345 NextSeq 1000/2000 Sequencing v1.1 Workflow Step Automations

Protocol	Step	Automation
NextSeq 1000/2000 Sequencing v2.0	Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.0)	• Calculate Volumes • Set Next Step
	Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.0)	• Set Next Step • Validate Single Input • Validate Reagent Cartridge Barcode • Retrieve Analysis Workflow Versions • Validate Run Setup and Create Planned Run
	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.0)	• Set Next Step
	Demultiplexing (NextSeq 1000/2000 Sequencing v2.0)	• Assign Demultiplex QC flags • Set Next Step

Terminology

The Illumina NextSeq 1000/2000 Integration Package v2.1 supports Clarity LIMS v5.3 and later.

As of Clarity LIMS v5, the term user-defined field (UDF) has been replaced with custom field in the user interface (the API resource is still called UDF). There are two types of custom fields:

- Master step fields—Configured on master steps. These fields only apply to the master step on which they are configured, and the steps derived from those master steps.
- Global fields—Configured on entities such as submitted sample, derived sample, measurement, etc. These fields apply to the entire Clarity LIMS system.

Step 1: Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.0)

In this step, pooled samples are diluted by the addition of RSB. Manually create a working pool based on the final loading concentration required.

Only create one pool per step for the Calculate Volume automation script to work properly.

Automations

The following automations are configured on the step:

- Calculate Volumes
- Set Next Step

Calculate Volumes

These automations are described in the order in which they are triggered at run time.

Select the Calculate Volumes button on the Record Details screen to trigger this automation automatically, which does the following actions:

- Sets RSB Volume for Pool (µl) field value to 24 for calculation purpose of the 2 nM intermediate library pool.
- Copies the Final Loading Concentration (pM) from step inputs to step outputs.

```
-exp 'step.::RSB Volume for Pool (µl):: = 24; output.::Final Loading
Concentration (pM):: = step.::Final Loading Concentration (pM)::'
```

- Calculate per sample volume required for each library to make the 2 nM intermediate library pool (24ul in volume).

```
-exp 'input.::Per Sample Volume (µl):: = ( 48/ input.::Molarity (nM):: );
step.::RSB Volume for Pool (µl):: -= input.::Per Sample Volume (µl)::'
```

- Calculate the volume required of the 2 nM library pool to be diluted further to the Final Loading Concentration (pM) with the Final Loading Volume (µl).

```
-exp 'step.::Library Pool Volume (µl):: = (step.::Final Loading Concentration
(pM):: * step.::Final Loading Volume (µl)::/2000); output.::RSB Volume (µl):: =
step.::Final Loading Volume (µl):: - step.::Library Pool Volume (µl)::'
```

- Uses the `NextSeq1K2K_Pool1.csv`, `NextSeq1K2K_Pool2.csv`, and `NextSeq1K2K_Pool3.csv` template files to generate a single CSV file containing information about the pool and the samples it contains. The generated file is stored in the Calculation File placeholder, in the Files section, for download.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} \
    script:driver_file_generator \
        -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NextSeq1K2K_
        Pool1.csv \
        -o 1.csv \
```

```
    script:driver_file_generator \
        -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NextSeq1K2K_
        Pool2.csv \
        -o 2.csv \
    script:driver_file_generator \
        -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NextSeq1K2K_
        Pool3.csv \
        -o 3.csv \
&& cat 1.csv 2.csv 3.csv > {compoundOutputFileLuid0}.csv
```

Set Next Step

Automatically triggered on exit of the Record Details screen, this automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol. The next step is Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.0):

```
nextStep = ::ADVANCE::
```

Master Step Fields

There are four fields defined on the Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.0) step.

Table 346 Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.0) Master Step Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Final Loading Concentration (pM)	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 650 • 750 • 1000 • 2000
Final Loading Volume (µl)	Numeric	• Required: Yes • Read Only: No	• Default: 24
Library Pool Volume (µl)	Numeric	• Required: No • Read Only: Yes	Not applicable
RSB Volume for Pool (µl)	Numeric	• Required: No • Read Only: Yes	Not applicable

Global Fields

The following table lists the global fields that are configured to display on the Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.0) step.

Table 347 Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.0) Global Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Final Loading Concentration (pM)	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 225 • 400 • Decimal Places Displayed: 0
RSB Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2

User Interaction

Refer to *NextSeq 1000/2000 Integration v2.1 Validation and Troubleshooting* on page 1697 for the details.

Step 2: Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.0)

In this step, scan the reagent cartridge barcode into Clarity LIMS, then manually place the working pool into the reagent cartridge for the NextSeq 1000/2000 run. This step validates the run setup and analysis information and generates the sample sheet file and/or creates a planned run on ICA, depending on the Run Mode selected.

Automations

The following automations are configured on the step:

- Retrieve Analysis Workflow Versions
- Set Next Step
- Validate Reagent Cartridge Barcode
- Validate Run Setup and Create Planned Run
- Validate Single Input

These automations are described in the order in which they are triggered at run time.

Validate Single Input

Automatically triggered at the beginning of the step, this automation checks that there is only one container input to the step.

```
script:validateSampleCount -min 1 -max 1
```

Validate Reagent Cartridge Barcode

Automatically triggered on exit of the Placement screen, this automation does the following action:

- Validates the reagent cartridge barcode to make sure it conforms to the barcode mask [A-Za-z]{2}[0-9]{7}-[A-Za-z0-9]{4}:

```
if (!output.container.name.matches( ::[A-Za-z]{2}[0-9]{7}-[A-Za-z0-9]{4}:: ) )
{fail ( ::Invalid Reagent Cartridge Barcode. Please verify and try again.:: ) }
```

Retrieve Analysis Workflow Versions

Automatically triggered upon entry of the Record Details screen, this automation:

Fetches the available analysis workflow versions from ICA and updates the preset values of both Local Analysis Workflow Versions & Cloud Analysis Workflow Versions UDFs.

```
/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/nextseq1k2k-
automation.jar script:retrieve_analysis_workflow_versions -i {stepURI:v2} -u
{username} -p {password} -l {compoundOutputFileLuid2}
```

Do not modify or disable this automation script as it breaks the integration.

Validate Run Setup and Create Planned Run

Automatically triggered when a button on the Record Details screen is selected, this automation does the following actions:

- Validates the parameters entered on the Record Details screen, which are used to set up the run and generate the sample sheet file and/or create the planned run on ICA.
  - Run Name can only contain alphanumeric, dash, underscore, or period characters. Spaces are not permitted.

```
if (!step.::Run Name::.matches(::[a-zA-Z0-9-_.]+::))
{ fail(::Run Name contains prohibited characters. Allowed characters are: a-z,
A-Z, 0-9, -, _, and .::) }
```

- Checks the Index Reads, Index Read 1, and Index Read 2 field values.
  - If Index Reads is No Index, Index Read 1 and Index Read 2 values must be zero (error results if it is otherwise).
  - If Index Reads is Single Index, Index Read 1 value must be greater than zero and Index Read 2 values must be zero (error results if it is otherwise).

  - If Index Reads is Dual Index, Index Read 1 and Index Read 2 values must be greater than zero (error results if it is otherwise).

```
if (step.::Index Reads:: == ::No Index::)
{ if (step.::Index Read 1:: != 0 || step.::Index Read 2:: != 0) { fail(::Index Read 1 and Index Read 2 must be 0 if the Index Reads is No Index.::) } }
else
{ if (step.::Index Reads:: == ::Single Index::)
{ if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) { fail(::Index Read 1 must be greater than 0 and Index Read 2 must be 0 if the Index Reads is Single Index.::) } }
else
{ if (step.::Index Read 1:: == 0 || step.::Index Read 2:: == 0) { fail(::Index Read 1 and Index Read 2 must be greater than 0 if the Index Reads is Dual Index.::) } } }
```

- Checks the Paired End, Read 2 Cycles, and Index Read 2 field values.
  - If Paired End is set to True and Read 2 Cycles value is zero, an error is generated.
  - If Paired End is set to False and Read 2 Cycles or Index Read 2 values are greater than zero, an error is generated.

```
if (step.::Paired End::.toBoolean())
{ if (step.::Read 2 Cycles:: == 0) { fail(::Read 2 Cycles must not be zero if it is Paired End read.::) }}
else
{if (step.::Read 2 Cycles:: != 0 || step.::Index Read 2:: != 0) {fail(::Read 2 Cycles and Index 2 Cycles must be 0 if it is not Paired End Read.:: )}}
```

- Checks the Adapter Sequence Read 1 and Adapter Sequence Read 2 field values.
  - Adapter Sequence Read 1 and Adapter Sequence Read 2 can only contain ACTG+ characters.

```
if (step.hasValue(::Adapter Sequence Read 1::) && !step.::Adapter Sequence Read 1::.matches(::[ACTG+]+::)) { fail(::Adapter Sequence Read 1 contains prohibited characters. Allowed characters are: ACTG+::) };
if (step.hasValue(::Adapter Sequence Read 2::) && !step.::Adapter Sequence Read 2::.matches(::[ACTG+]+::)) { fail(::Adapter Sequence Read 2 contains prohibited characters. Allowed characters are: ACTG+::) }
```

- Checks Override Cycles field value.
  - Override Cycles can only contain Y, N, I, U, 0–9, and semicolon characters.

```
if (step.hasValue(::Override Cycles::) && !step.::Override Cycles::.matches(::[YNIU0-9;]+::))
{ fail(::Override Cycles contains prohibited characters. Allowed characters are: Y, N, I, U, 0-9 and ;. Example: N1Y150;I8;I7N1;Y141U10.::) }
```


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- Sets Cloud Analysis Workflow Versions, Local Analysis Workflow Versions and FASTQ Compression Format to None when Analysis Workflow is "None".

```
if (step.::Analysis Workflow:: == ::None::) { step.::Cloud Analysis Workflow
Versions:: = ::None::; step.::Local Analysis Workflow Versions:: = ::None::;
step.::FASTQ Compression Format:: = ::None::; }
```

- When Analysis Workflow is GenerateFASTQ and Run Mode is Cloud:
  - Set Local Analysis Workflow Versions to None.
  - If Cloud Analysis Workflow Versions is None throw the following error message: Invalid option selected for Cloud Analysis Workflow Versions field.

```
if (step.::Analysis Workflow:: == ::GenerateFASTQ:: && step.::Run Mode:: ==
::Cloud::) { step.::Local Analysis Workflow Versions:: = ::None::; if
(step.::Cloud Analysis Workflow Versions:: == ::None::) { fail(::Invalid option
selected for Cloud Analysis Workflow Versions field.::); }};
```

  - Due to automation script limitation, a validation rule resides in the backend service to perform the following action: If Cloud Analysis Workflow Versions is 3.7.4 or later, set FASTQ Compression Format to gzip.
- When Analysis Workflow is GenerateFASTQ and Run Mode is Local or Hybrid:
  - Set Cloud Analysis Workflow Versions to None.
  - If Local Analysis Workflow Versions is None throw an error Invalid option selected for Local Analysis Workflow Versions field.

```
if (step.::Analysis Workflow:: == ::GenerateFASTQ:: && (step.::Run Mode:: ==
::Local:: || step.::Run Mode:: == ::Hybrid::)) { step.::Cloud Analysis Workflow
Versions:: = ::None::; if (step.::Local Analysis Workflow Versions:: ==
::None::) { fail(::Invalid option selected for Local Analysis Workflow Versions
field.::); }};
```

  - Due to automation script limitation, a validation rule resides in the backend service to perform the following action: If Local Analysis Workflow Versions is 3.7.4 or above, throw an error if FASTQ Compression Format is "None".
- Generates the sample sheet and/or creates the planned run on ICA.
  - Sample sheet is attached to the step.

```
/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/nextseq1k2k-
automation.jar
script:generate_v2_sample_sheet -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid2} -c {compoundOutputFileLuid0}
```


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- There is backend validation of the sample sheet content to make sure that the sample sheet is valid to set up a Local Mode run or ready to be consumed by DRAGEN applications. Refer to *NextSeq 1000/2000 Integration v2.1 Validation and Troubleshooting* on page 1697 for submitted sample name character restriction.

Set Next Step

Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol—AUTOMATED - Sequencing Run NextSeq 1000/2000 Sequencing v2.0.

```
nextStep = ::ADVANCE::
```

Master Step Fields

There are 24 fields defined on the Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.0) step. These fields are required for sample sheet generation and planned run creation on ICA.

Table 348 Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.0) Master Step Fields Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Run Name	Text	• Required: Yes • Read Only: No	Not applicable
Instrument Type	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• NextSeq1000 • NextSeq2000
Run Mode	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• Local • Hybrid • Cloud
Paired End	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• True • False
Read 1 Cycles	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 151 • 101 • 51

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Read 2 Cycles	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 151 • 101 • 51
Index Reads	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• No Index • Single Index • Dual Index
Index Read 1	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 0 • 6 • 8
Index Read 2	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 0 • 6 • 8
Analysis Workflow	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• GenerateFASTQ • None
Adapter Sequence Read 1	Text	• Required: No • Read Only: No	Not applicable
Adapter Sequence Read 2	Text	• Required: No • Read Only: No	Not applicable
Barcode Mismatches Index 1	Numeric Dropdown	• Required: No • Read Only: No • Custom Entries: No	• 0 • 1 • 2
Barcode Mismatches Index 2	Numeric Dropdown	• Required: No • Read Only: No • Custom Entries: No	• 0 • 1 • 2
Override Cycles	Text	• Required: No • Read Only: No	Not applicable
Cloud Run ID	Text	• Required: No • Read Only: Yes	• Hidden - Used in creating planned run in ICA

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Use Custom Index Read 1 Primer	Toggle Switch	• Required: No • Read Only: No	• Hidden Custom primer option is not supported at the moment. Do not enable as it causes integration to break.
Use Custom Index Read 2 Primer	Toggle Switch	• Required: No • Read Only: No	• Hidden Custom primer option is not supported at the moment. Do not enable as it causes integration to break.
Use Custom Read 1 Primer	Toggle Switch	• Required: No • Read Only: No	• Hidden Custom primer option is not supported at the moment. Do not enable as it causes integration to break.

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Use Custom Read 2 Primer	Toggle Switch	• Required: No • Read Only: No	• Hidden Custom primer option is not supported at the moment. Do not enable as it causes integration to break.
Instructions	Multi-Line Text	• Required: No • Read Only: Yes	• Default: If GenerateFASTQ analysis is required, select the DRAGEN version in Cloud Analysis Workflow Versions (for Cloud run mode) or Local Analysis Workflow Versions (for Local/Hybrid run mode) custom fields.

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Local Analysis Workflow Versions	Text Dropdown	• Required: No • Read Only: No • Custom Entries: No	• None (default value) Do not modify the configuration of Local Analysis Workflow Versions and Cloud Analysis Workflow Versions UDF as it breaks the integration.
Cloud Analysis Workflow Versions	Text Dropdown	• Required: No • Read Only: No • Custom Entries: No	• None (default value) Do not modify the configuration of Local Analysis Workflow Versions and Cloud Analysis Workflow Versions UDF as it breaks the integration.
FASTQ Compression Format	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• GZIP (default value) • DRAGEN • None

User Interaction

Refer to *NextSeq 1000/2000 Integration v2.1 Validation and Troubleshooting* on page 1697 for details.

Step 3: AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.0)

This step is fully automated.

The integrations start and complete the step automatically and data from the run is parsed back to Clarity LIMS. No user interaction is required. In this step, the pooled samples in the reagent cartridge are sequenced on the NextSeq 1000/2000 instrument.

Do not add samples to the Ice Bucket or start or complete the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.0) step. The integration does this step automatically.

Automations

The following automations are configured on the step:

- Set Next Step
  - The Set Next Step automation is the only automation configured on this step. It automatically sets the samples to advance to the next step—Demultiplexing (NextSeq 1000/2000 Sequencing v2.0).

```
nextStep = ::ADVANCE::
```

Master Step Fields

There are 16 fields defined on the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.0) step. These fields are used to display the run status and sequencing run and analysis configuration parsed from the `RunParameters.xml` file of the sequencing run.

Do not modify the name of any of the master step fields. It causes the integration to break.

See the following table for how some of these step fields map to the fields on the `RunParameters.xml` file.

Table 349 Run Parameters and Corresponding Clarity LIMS Step Fields

Master Step Field	RunParameters.xml Field
Current Cycle	Calculated based on CompletedCycles Field
Current Read	Calculated based on CompletedCycles field against PlannedCycles
Flow Cell ID	FlowCellSerialNumber
Flow Cell Lot Number	FlowCellLotNumber
Instrument Control Software Version	ApplicationVersion
Instrument ID	InstrumentSerialNumber
Output Folder	OutputFolder
Reagent Cartridge ID	CartridgeSerialNumber
Reagent Cartridge Lot Number	CartridgeLotNumber

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Master Step Field	RunParameters.xml Field
RTA Version	RtaVersion
Run Name	ExperimentName
Secondary Analysis Workflow	SecondaryAnalysisWorkflow

Global Fields

The following global fields are used to capture the run metrics in Clarity LIMS.

- % Bases >=Q30 R1
- % Bases >=Q30 R2
- % Error Rate R1
- % Error Rate R2
- Yield (Gb) R1
- Yield (Gb) R2
- Reads PF R1
- Reads PF R2
- %PF R1
- %PF R2
- % Aligned R1
- % Aligned R2
- % Phasing R1
- % Phasing R2
- % PrePhasing R1
- % PrePhasing R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- Cluster Density R1
- Cluster Density R2

At the end of this step, the pool of samples undergoes either one of the two following actions:

1. If the Analysis Workflow UDF in Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.0) step is set to GenerateFASTQ, the pool automatically advances to and is queued for the Demultiplexing (NextSeq 1000/2000 Sequencing v2.0) step.

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2. If the Analysis Workflow UDF in Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.0) step is set to None, the pool automatically completes the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.0) step and gets removed from the workflow.

Step 4: Demultiplexing (NextSeq 1000/2000 Sequencing v2.0)

This step is a semiautomated step.

The integration starts the step automatically and demultiplexing data from the GenerateFASTQ secondary analysis is parsed back to Clarity LIMS. Then review the demultiplexing result parsed into Clarity LIMS, assign QC flags, and complete the step.

For the demultiplexing information in the Sample Details table to display correctly, the following conditions must be met:

- The DRAGEN version must be earlier than v3.9.3.
- The samples in the run must not repeat.
- The run must be for the single lane flow cell (P1 or P2).

Do not add samples to the Ice Bucket or start or complete the step. The integration completes these actions automatically.

Automations

The following automations are configured on the step:

- Assign Demultiplexing QC flags—Automatically assigns QC flags based on the criteria set on the step fields.
- Set Next Step—Automatically sets the next step to Advance and the samples complete the protocol.

Master Step Fields

There are eight fields defined on the Demultiplexing (NextSeq 1000/2000 Sequencing v2.0) step.

The following table lists the master step fields that are configured on the Demultiplexing (NextSeq 1000/2000 Sequencing v2.0) step.

Table 350 Master Step Field Configuration for Demultiplexing (NextSeq 1000/2000 Sequencing v2.0) Step

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Criteria 1 - Source Data Field	Text Dropdown	• Required: No • Read Only: No • Custom Entries: Yes	• # Reads • # Perfect Index Reads • # One Mismatch Index Reads • # of >= Q30 Bases (PF)
Criteria 2 - Source Data Field	Text Dropdown	• Required: No • Read Only: No • Custom Entries: Yes	• # Reads • # Perfect Index Reads • # One Mismatch Index Reads • # of >= Q30 Bases (PF)
Criteria 1 - Operator	Text Dropdown	• Required: No • Read Only: No • Custom Entries: Yes	• >= • <= • = • !=
Criteria 2 - Operator	Text Dropdown	• Required: No • Read Only: No • Custom Entries: Yes	• >= • <= • = • !=
Criteria 1 - Threshold Value	Numeric	• Required: No • Read Only: No	• Valid integer value
Criteria 2 - Threshold Value	Numeric	• Required: No • Read Only: No	• Valid integer value
Log	Multiline Text	• Required: No • Read Only: Yes	Not applicable
Reagent Cartridge ID	Text	• Required: No • Read Only: Yes	• Hidden • Used for parsing demultiplexing results

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Global Fields

There are four fields defined on the Demultiplexing (NextSeq 1000/2000 Sequencing v2.0) step. which are used to display the demultiplexing result metrics for individual library in the sample pool.

The following table lists the global fields that are configured on the Demultiplexing (NextSeq 1000/2000 Sequencing v2.0) step.

Table 351 Global Field Configuration for Demultiplexing (NextSeq 1000/2000 Sequencing v2.0) Step

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
# of >= Q30 Bases (PF)	Numeric	• Required: No • Read Only: Yes	Not applicable
# One Mismatch Index Reads	Numeric	• Required: No • Read Only: Yes	Not applicable
# Perfect Index Reads	Numeric	• Required: No • Read Only: Yes	Not applicable
# Reads	Numeric	• Required: No • Read Only: Yes	Not applicable

User Interaction

Refer to *NextSeq 1000/2000 Integration v2.1 Validation and Troubleshooting* on page 1697 for details.

How the NextSeq 1000/2000 Integration Works

Sample Sheet Generation and Planned Run Creation

On the Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.0) step, when the Validate Run Setup and Create Planned Run automation is triggered, all the run and analysis parameters that were entered in the Run Details screen are sent to Illumina Connected Analytics (ICA). ICA validates the run and analysis configuration and sends an error message to Clarity LIMS if the validation failed. If the validation passes, ICA generates the sample sheet (and sends back to Clarity LIMS) and/or creates a planned run depending on the Run Mode selected.

For Local run mode, only the sample sheet is generated and stored on Clarity LIMS. The sample sheet contains all the run and analysis configuration required to start the run on the instrument.

For Hybrid/Cloud run mode, a planned run is created on ICA. This planned run contains all the run and analysis configuration required to start the run on the instrument. The sample sheet is also generated and stored on Clarity LIMS for reference purpose.

Refer to *Step-by-Step Instruction to Start A Sequencing Run On Instrument With Different Run Modes on page 1695* for more details on how to start a run using different run mode.

Run Status, Primary Metrics and Analysis Results Parsing and Recording

After the sequencing run is started on the instrument, NextSeq 1000/2000 Control Software notifies BaseSpace Sequence Hub (BSSH) that the sequencing run has been started.

BSSH then does the following actions:

- For Local run mode, create a new run on the BSSH database with the run configuration information received from NextSeq 1000/2000 Control Software.
- For Hybrid/Cloud run mode, create a new run on the BSSH database by copying the run configuration information from the planned run created in ICA, using a special Cloud Run ID received from NextSeq 1000/2000 Control Software.

After the run has been created on BSSH, a run update event message that carries the RunStarted equivalent message is created on BSSH and delivered through ICA and, eventually, to Clarity LIMS SQS queue service. The message is grabbed and processed by SIS integration service.

Based on the reagent cartridge barcode information in the event message (see the following example), SIS looks in the queue of AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.0) step for a container with the same reagent cartridge barcode. When identified, the step is started automatically and the Run Status UDF on Record Details screen is updated to RunStarted. SIS then updates all the other step UDFs (eg, Current Read, Output Folder etc.) by parsing the details from the `RunParameters.xml` uploaded to BSSH by NextSeq 1000/2000 Control Software.

```
#Example of an run update event
{
    "acl": [
       "wid:61dc14e8-d823-3bd1-bea3-66f9c8388254"
    ],
    "apiUrl":
    "https://api.basespace.illumina.com/v2/runs/r.ZvTfDXsNhUyh2kA5S4Zewg",
    "dateModified": "2020-03-26T09:19:58.5546497Z",
    "flowcellBarcode": "11M11COMC",
    "gdsFolderPath": "/Runs/200326_MP2-15_87_11M11COMC_r.ZvTfDXsNhUyh2kA5S4Zewg",
    "gdsVolumeName": "bssh.61dc14e8d8233bd1bea366f9c8388254",
    "id": "r.ZvTfDXsNhUyh2kA5S4Zewg",
    "instrumentRunId": "200326_MP2-15_87_11M11COMC",
    "name": "200326_MP2-15_87_11M11COMC",
```


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```
    "reagentBarcode": "EC0001990-EC5",
    "sampleSheetName": "SampleSheet.csv",
    "status": "Running",
    "v1pre3Id": "194424236"
}
```


NextSeq 1000/2000 Control Software continues to notify BSSH of any run status change throughout the course of the sequencing run, and the same run update event delivery process follows.

The following table lists out all the run status and how they are being handled by SIS integration service.

Table 352 Run Status and How SIS Integration Service Handles the Status

Run Status	Shown in Step	Details

RunStarted	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.0)	• Run Status UDF is updated to RunStarted.
		• All other step UDFs are updated based on RunParameters.xml detail obtained from BSSH.
		• Reagent cartridge lot information received from BSSH is compared against the details of the preaccessioned reagent cartridge on Clarity LIMS.
		  ◦ If the expiration date is different, it is updated to the date received from BSSH.
		  ◦ If reagent cartridge is not found, it is created and set to Active.
		  ◦ If reagent cartridge is not in Active status, it is set to Active.
		• Multiline Sequencing Log UDF is updated.

Run Status	Shown in Step	Details
RunCompletedSuccessfully	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.0)	• Run Status UDF is updated to RunCompletedSuccessfully • Current Read and Current Cycle UDFs is updated to the final cycle number and read number based on `RunParameters.xml` detail obtained from BSSH. • Sequencing run result (primary metrics) is downloaded from BSSH and the fields in Sample Details table (eg, % Error Rate R1, % Phasing, etc.) are updated with the values. • Multiline Sequencing Log UDF is updated. • SIS autocompletes the step and routes the samples in the reagent cartridge container to the next step (ie, Demultiplexing (NextSeq 1000/2000 Sequencing v2.0) step if GenerateFastQ is selected as the Analysis Workflow in Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.0) step). Otherwise, the step is completed automatically and the samples are removed from the workflow.

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Run Status	Shown in Step	Details
RunErroredOut	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.0)	• Run Status UDF is updated to RunErroredOut • Current Read and Current Cycle UDFs are updated to the final cycle number and read number that the run stopped based on RunParameters.xml detail obtained from BSSH. • Sequencing run result (primary metrics) is downloaded from BSSH and the fields in Sample Details table (eg, % Error Rate R1, % Phasing, etc.) are updated with the values. • Multiline Sequencing Log UDF is updated. • SIS does not autocomplete the step. Complete the step manually.
RunAbortedByUser	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.0)	• Run Status UDF is updated to RunAbortedByUser • Current Read and Current Cycle UDFs are updated to the final cycle number and read number that was aborted based on RunParameters.xml details obtained from BSSH. • Sequencing run result (primary metrics) is downloaded from BSSH and the fields in Sample Details table (eg, % Error Rate R1, % Phasing, etc.) are updated with the values. • Multiline Sequencing Log UDF is updated. • SIS does not autocomplete the step. Complete the step manually.
AnalysisStarted	Demultiplexing (NextSeq 1000/2000 Sequencing v2.0)	• SIS starts the step automatically for the reagent cartridge container. • Multiline Sequencing Log UDF is updated.

Run Status	Shown in Step	Details
AnalysisCompleted	Demultiplexing (NextSeq 1000/2000 Sequencing v2.0)	• SIS downloads the `Demultiplex_stats.csv` file, which contains the demultiplexing result from BSSH. • The file is parsed and details are recorded in the Sample Details table for each library in the library pool. • Multiline Sequencing Log UDF is updated. • SIS does not autocomplete the step. Assign the QC flag to the individual library and manually complete the step.

Step-by-Step Instruction to Start A Sequencing Run On Instrument With Different Run Modes

Local Run Mode

1. On the NextSeq 1000/2000 Control Software interface, select the menu button and select Settings.
2. Select Local Run Setup and Proactive, Run Monitoring and Storage*.
3. [Optional] Enter the default output folder location.
4. Select Save.
5. On the main screen, select Start.
6. Sign in to the BaseSpace Sequence Hub (BSSH) account that was used during NextSeq 1000/2000 Integration package installation setup.
   a. Select the workgroup (if applicable).
   b. Select Next.
7. Select Choose, and a file browser pops up.
8. Browse and select the sample sheet generated during the Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.0) step.
   When selected, the sample sheet has its content validated. After the validation passes, run and analysis configuration is parsed and autopopulated in the next page.
9. Select Review to review the sequencing run settings.
10. Select Load and manually load the reagent cartridge and flow cell.
11. Select Sequence and the pre-run check proceeds.
    After the pre-run check passes, the sequencing run starts automatically.

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Hybrid or Cloud Run Mode

1. On the NextSeq 1000/2000 Control Software interface, select the menu button and select Settings.
2. Select Online Run Setup and Proactive, Run Monitoring and Storage*.
3. [Optional] Enter the default output folder location.
4. Select Save.
5. On the main screen, select Start.
6. Sign into the BaseSpace Sequence Hub (BSSH) account that was used during NextSeq 1000/2000 Integration package installation setup.
   a. Select the workgroup (if applicable).
   b. Select Next.
   Planned runs that were created in the Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.0) step, using Hybrid or Cloud run mode, display as options.
7. Select the run and select Review to review the sequencing run settings.
8. Select Load and manually load the reagent cartridge and flow cell.
9. Select Sequence and the pre-run check proceeds.
   After the pre-run check passes, the sequencing run starts automatically.

Components Installed

The following sections describe the various components that are installed by default as part of this integration. These components include files, properties, reagent categories/label groups, reagent kits, control types, and containers. Information on installed workflows, protocols, steps, and automation points is provided in the *Workflows, Protocols, and Steps* on page 1672 section.

Reagent Categories/Label Groups

- TruSeq HT Adapters v2 (D7-D5)

Reagent Kits

- Resuspension Buffer (RSB)
- NextSeq 1000/2000 Reagent Cartridge

Container Types

- Tube
- NextSeq 1000/2000 Reagent Cartridge

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Control Types

- PhiX v3

This integration supports:

- NextSeq 1000/2000 Reagent Cartridge with barcode provided in the format [A-Za-z]{2}[0-9]{7}-[A-Za-z0-9]{4}
  - Example: EC1234567-EC03

Rules and Constraints

- The workflow configuration contains several validation checks. To make sure that the calculations work properly, it is important that you do not disable any of this validation logic. The validation checks determine:
  - Which samples, and how many, can enter each step together.
  - Which samples, and how many, can be pooled together.
- The NextSeq 1000/2000 reagent cartridge barcode must be unique. There should not be multiple NextSeq 1000/2000 reagent cartridge containers in the system with the same name.
- Reagent labels (indexes) must be unique.
- One library pool can only contain one library or control with no label (index).
- Do not manually start or complete the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.0) step. This step is a fully automated step, and the SIS integration service does not update samples correctly if they have been manually started.
- Do not manually start the Demultiplexing (NextSeq 1000/2000 Sequencing v2.0) step. This step is semiautomated, and the SIS integration service does not update the demultiplexing result correctly if they have been manually started.
- For the automated run to start successfully, select Validate Run Setup and Create Planned Run in the Load to Reagent Cartridge step.

NextSeq 1000/2000 Integration v2.1 Validation and Troubleshooting

This section explains how to validate the installation of the Illumina NextSeq 1000/2000 Integration Package v2.1.

The validation process involves:

- Running samples through the Library Prep Validation v2.0.3 workflow.
  - The workflow contains a single-step protocol that models the library prep workflow required to produce libraries tagged with index sequences. At the end of the step, these libraries are routed to NextSeq 1000/2000 Sequencing v2.0 workflow.
- Running the libraries through the NextSeq 1000/2000 Sequencing v2.0 workflow. This process validates the following information:

- Successful sequential step advancement of samples in the following steps:
  1. Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.0)
  2. Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.0)
  3. AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.0)
  4. [Optional] Demultiplexing (NextSeq 1000/2000 Sequencing v2.0)
- Automatic validation of run setup information before sample sheet generation or planned run creation.
- Automated generation of a sample sheet. The sample sheet is used to start the sequencing run on NextSeq 1000/2000 Control Software via Local run mode.
- Automated creation of a planned run on Illumina Connected Analytics (ICA). The planned run created is retrieved by NextSeq 1000/2000 Control Software to start run via Hybrid or Cloud run mode.
- Automated tracking of the NextSeq 1000/2000 sequencing run and parsing of run statistics from BaseSpace Sequence Hub (BSSH) into Clarity LIMS using SIS Integration Service.
- Automated tracking of the secondary analysis using DRAGEN (up to BCL Convert only) and parsing of demultiplexing metrics from BaseSpace Sequence Hub (BSSH) into Clarity LIMS using SIS integration service.

Before running the validation steps below, these steps assume that the NextSeq 1000/2000 Sequencing v2.0 Integration Package is installed and you have imported the default Clarity LIMS configuration.

For information on how the integration works, refer to *NextSeq 1000/2000 Integration v2.1 Configuration and User Interaction* on page 1670.

Activate Workflow, Create Project, Add and Assign Samples

The following steps set up Clarity LIMS in preparation for running samples through the Library Prep Validation v2.0.3 and NextSeq 1000/2000 Sequencing v2.0 workflows.

1. In the Configuration tab of Clarity LIMS under Workflows, activate both the Library Prep Validation v2.0.3 and NextSeq 1000/2000 Sequencing v2.0 workflows.
2. On the Projects and Samples screen, create a project and add samples to it.

   Use only alphanumeric, dash, and underscore characters in the submitted sample names. Failing to do so causes sample sheet validation failure in Load To Reagent Cartridge step.

3. Assign the samples to the Library Prep Validation v2.0.3 workflow.

Library Prep Protocol: Library Prep Validation v2.0.3

This single-step protocol models the library prep required to produce libraries tagged with index sequences that are ready for the NextSeq 1000/2000 Sequencing v2.0 workflow.

Run Library Prep Validation v2.0.3 Step

1. In Lab View, locate the Library Prep Validation v2.0.3 protocol. The samples are queued for the Library Prep Validation v2.0.3 step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select the label groups to use.
   - TruSeq HT Adapters v2 (D7-D5) is the default label group delivered by Library Prep Validation v2.0.3 protocol.
   - For other reagent labels (indexes), manually create them in the Configuration screen under Consumables → Labels.

   > For more information on how to create reagent label group, refer to the *Add and Configure Labels and Label Groups* section in the Clarity LIMS Core Functionality documentation.

4. Select Begin Work.
5. On the Placement screen:
   a. In the Placed Samples area in the Container field, scan or enter the barcode of the destination container.
   b. Select the samples from the Samples to be Placed area, then drag them over to the container wells on the right.
   c. Select Add Labels.
6. On the Add Labels screen, the Labels to Add list is populated with labels from the TruSeq HT Adapters v2 (D7-D5) label group. This label group is the only one configured in the out-of-the-box integration. It is used by default in this step.
   a. Select labels on the left, and drag them over to the container on the right to assign them to placed samples.
   b. Select Record Details.
7. On the Record Details screen:
   a. In the Sample Details table, enter values for Molarity (nM) for all the individual sample. Type values individually or use the copy/paste functionality to enter multiple values from a spreadsheet.
      - Molarity (nM) values are used in Calculate Volume automation script in Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.1) step.

      > If Molarity (nM) values are not entered, the Calculate Volume automation script does not work properly.

   b. The Sequencing Instrument column indicates the instrument to be used for the run. Select NextSeq 1000/2000.
   c. Populate the fields in this column using one of the following methods:

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- To populate fields individually for each sample, select from the drop-down list in each row.
- To populate fields all at one time, use the Copy and Paste buttons to enter multiple values from a spreadsheet (see the following note).

Alternatively, select inside the table and press Ctrl + A to select all rows. At the top of the table, select Sequencing Instrument, then select NextSeq 1000/2000 from the adjacent drop-down list. Select Apply.

If you are working with many samples, you can use the copy/paste functionality to enter multiple values from a spreadsheet.

a. Select the Copy button to copy the sample information from Clarity LIMS to the clipboard.
b. Open a blank Excel document and paste the contents of the clipboard into the spreadsheet.
c. In the spreadsheet, enter the Molarity (nM) and Sequencing Instrument values. Select the entire spreadsheet, including the headers, (Ctrl + A) and copy (Ctrl + C) the information to the clipboard.
d. In Clarity LIMS, select the Paste button. Paste the contents of the clipboard (Ctrl + V) into the Update Table window and then select Update to paste into the Sample Details table.

8. Select Next Steps.
   This selection triggers the Next Steps automation, which sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step successfully.
9. On the Assign Next Steps screen in the Samples table, the Next Step for all samples is prepopulated with Remove from workflow.

   Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.

10. Select Finish Step.
    On exit from the step, the Routing Script automation is triggered. This automation assigns samples to the first step of the NextSeq 1000/2000 Sequencing v2.0 workflow, which is Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.0) step.

NextSeq 1000/2000 Sequencing v2.0 Protocol

Step 1: Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.0)

1. In Lab View, locate the NextSeq 1000/2000 Sequencing v2.0 protocol. The samples are queued for the Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.0) step.

2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Pooling screen:
   a. Create a pool by dragging samples into the Pool Creator.
   b. Type a name for the pool or accept the default name (Pool #1).
   c. Select Record Details.
5. On the Record Details screen, the Reagent Lot Tracking section tracks the Resuspension Buffer (RSB) lot information used in the step. You might need to add and activate lots for RSB.
   To add and activate reagent lots:
   a. In a new browser tab, open an additional instance of Clarity LIMS.
   b. Navigate to the Consumables configuration screen.
   c. Select the RSB reagent kit and select New Lot.
   d. Enter the lot details and select Save.
   e. Set the Status of Reagent Lot toggle switch to Active.
6. In the Reagent Lot Tracking section, select from the active lots displayed in the drop-down list.
7. The Step Details area contains two required fields:
   - Final Loading Concentration (pM)—The value entered in this field is the recommended final loading concentration from Illumina NextSeq 1000/2000 Instrument System Guide depending on the library type. There is a default drop-down list of 650, 750, 1000 and 2000. A custom value is acceptable.
   - Final Loading Volume (µl)—The value in this field is the final loading volume of the pool into the reagent cartridge. The field is prepopulated with the configured default value, 24 µl, which is recommended by Illumina NextSeq 1000/2000 Instrument System Guide. However, it can be edited if more volume is necessary.
8. Select Calculate Volumes. This selection triggers the Calculate Volumes automation. This automation calculates the volume required for each library to form a pool that has the concentration and volume specified in the step details fields.
   The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains volume information of each of the samples and RSB buffer to add to the pool. Select the file to download it, then open it in Excel.
9. In the Sample Details table, select the pool icon to view details on the pool composition.
10. Select Next Steps. This selection triggers the Set Next Step automation, which sets the next step for samples to ADVANCE, advancing them to the next step in the protocol. The next step is Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.0).
    On the Assign Next Steps screen, the next step for samples is already set to the next step in the workflow. The next step is Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.0).
11. Select Finish Step.

At the end of this step, the pool of samples automatically advances to Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.0) step.

Step 2: Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.0)

1. In Lab View, locate the NextSeq 1000/2000 Sequencing v2.0 protocol. The pool of samples is queued for the Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.0) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
   The Validate Single Input automation is triggered. This automation checks that there is only one container input to the step.
4. On the Placement screen:
   a. Drag the pool into the NextSeq 1000/2000 Reagent Cartridge in the Placed Samples area.
   b. Scan or type the barcode of the reagent cartridge into the NextSeq 1000/2000 Reagent Cartridge field.
   c. Select Record Details.
   On exit of the Placement screen, the Validate Reagent Cartridge Barcode automation checks that the reagent cartridge barcode conforms to the barcode mask [A-Za-z]{2}[0-9]{7}-[A-Za-z0-9]{4}. If not, an error message displays.
   On entry to the Record Details screen, the Retrieve Analysis Workflow Versions automation fetches the available analysis workflow versions from ICA and update the preset values of both Local Analysis Workflow Versions & Cloud Analysis Workflow Versions UDF
5. On the Record Details screen, the Reagent LotTracking section tracks the NextSeq 1000/2000 Reagent Cartridge lot information used in the step. Follow the steps in Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.0) step if you need to add a new lot.
6. In the Reagent Lot Tracking section, select from the active lots displayed in the drop-down list.
7. The fields displayed on the Record Details screen are used to create planned run and generate the sample sheet file.
   - Run Name—Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.
   - Instrument Type—Select from preset options: NextSeq 1000, NextSeq 2000.
   - Run Mode—Select from preset options: Local, Hybrid, Cloud. See *Run Mode Details* on page 1704 for more information on different run mode.
   - Paired End—Select from preset options: True or False.
   - Read 1 Cycles—Select from preset options (151, 101, 51) or type a custom value.
   - Read 2 Cycles—Select from preset options (151, 101, 51) or type a custom value.
   - Index Reads—Select from preset options: No Index, Single Index, Dual Index.

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- Index Read 1—Select from preset options (0, 6, 8) or type a custom value.
- Index Read 2—Select from preset options (0, 6, 8) or type a custom value.
- Analysis Workflow—Select from preset options: GenerateFASTQ or None. See *Analysis Workflow Details* on page 1704 for more information.
- Local Analysis Workflow Versions—[Optional] Enter the Read 1 (defaulted to None). Only mandatory when Run Mode = Local/Hybrid and Analysis Workflow = GenerateFASTQ. Select according to the DRAGEN version installed on the instrument.
- Cloud Analysis Workflow Versions—[Optional] (defaulted to None) Only mandatory when Run Mode = Cloud and Analysis Workflow = GenerateFASTQ. Select the cloud DRAGEN version to be used.
- FASTQ Compression Format—Select gzip or DRAGEN when Run Mode = Local/Hybrid, Analysis Workflow = GenerateFASTQ and Local Analysis Workflow Version >= 3.7.4. For Run Mode = Cloud, Analysis Workflow = GenerateFASTQ and Local Analysis Workflow Version >= 3.7.4, it is set to gzip automatically. Select None otherwise.
- Adapter Sequence Read 1—[Optional] Enter the Read 1 adapter sequence of the index adapter kit.
- Adapter Sequence Read 2—[Optional] Enter the Read 2 adapter sequence of the index adapter kit.
- Barcode Mismatches Index 1—[Optional] Select from preset options: 0, 1, 2. Leave it blank if you are unsure.
- Barcode Mismatches Index 2—[Optional] Select from preset options: 0, 1, 2. Leave it blank if you are unsure.
- Override Cycles—[Optional] String used to specify UMI cycles and mask out cycles of a read, eg, N1Y150;I8;I7N1;Y141U10. Leave it blank if you are unsure.

8. On the Record Details screen, select Validate Run Setup and Create Planned Run. This selection triggers the automation script, which does the following actions:
   - Validates the parameters entered on the Record Details screen.
   - Create the planned run and send it to Illumina Connected Analytics (ICA) if Run Mode is Hybrid or Cloud.
   - Generates the sample sheet and attaches it to the placeholder in the Files area of the Record Details screen for all Run Mode types.
9. Select Next Steps.
   On the Assign Next Steps screen, the next step for samples is set to the next step in the workflow. The next step is AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.0).
10. Select Finish Step.

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At the end of this step, the pool of samples automatically advances to and is queued for the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.0) step. The sequencing run is ready to be started. For details on how to start the sequencing run for different run modes, refer to *NextSeq 1000/2000 Integration v2.1 Configuration and User Interaction* on page 1670.

Run Mode Details

The run mode applies to all runs and determines how the run and analysis parameters are configured and how to analyze data.

- Local Mode—NextSeq 1000/2000 Integration service generates the sample sheet. Run/Analysis configuration is imported manually into NextSeq 1000/2000 instrument via a sample sheet to start the run. The downstream secondary analysis is done using the onboard DRAGEN module.
- Hybrid Mode—NextSeq 1000/2000 Integration service sends the run/analysis configuration to Illumina Connected Analytics Platform (ICA). Run/Analysis configuration is downloaded to NextSeq 1000/2000 instrument via ICA to start the run. The downstream secondary analysis is done using the onboard DRAGEN module.
- Cloud Mode—NextSeq 1000/2000 Integration service sends the run/analysis configuration to Illumina Connected Analytics (ICA). Run/Analysis configuration is downloaded to NextSeq 1000/2000 instrument via ICA to start the run. The downstream secondary analysis is performed in the cloud.

Analysis Workflow Details

The analysis workflow selection determines if the sample is advanced to Demultiplexing (NextSeq 1000/2000 Sequencing v2.0) step or completed at AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.0) step.

- GenerateFASTQ—After the sequencing run completes, NextSeq 1000/2000 integration service automatically completes the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.0) step. Samples are advanced to the Demultiplexing (NextSeq 1000/2000 Sequencing v2.0) step.
- None—None is selected to skip Demultiplexing (NextSeq 1000/2000 Sequencing v2.0) step. NextSeq 1000/2000 integration service automatically completes the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.0) and removes the samples from the workflow.

Step 3: AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.0)

This step is fully automated.

Do not add samples to the Ice Bucket or start or complete the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.0) step. The integration does this action automatically.

The integration starts the step automatically and data from the run is parsed back to Clarity LIMS. No user interaction is required. However, you can open and review the various stages of the step in Clarity LIMS. Do not perform any action when reviewing the data.

Read summary metrics are recorded for the library pool in the Step Details section and the Sample Details table.

Values are populated in the following step UDFs/master step fields:

Run Name	Run Status	Output Folder
Current Read	Current Cycle	Secondary Analysis Workflow
Flow Cell ID	Flow Cell Lot Number	Reagent Cartridge ID
Reagent Cartridge Lot Number	Instrument Platform	Instrument Type
Instrument ID	Instrument Control Software Version	RTA Version
Sequencing Log		

The summary metrics (per run level) populate in the following UDFs/global fields.

- % Bases >=Q30 R1
- % Bases >=Q30 R2
- % Error Rate R1
- % Error Rate R2
- Yield (Gb) R1
- Yield (Gb) R2
- Reads PF R1
- Reads PF R2
- %PF R1
- %PF R2
- % Aligned R1
- % Aligned R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- Intensity Cycle 1 R1

- Intensity Cycle 1 R2
- Cluster Density R1
- Cluster Density R2

At the end of this step, the pool of samples undergoes either one of the following two actions:

1. If the Analysis Workflow UDF in Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.0) step is set to GenerateFASTQ, the pool automatically advances to and is queued for the Demultiplexing (NextSeq 1000/2000 Sequencing v2.0) step.
2. If the Analysis Workflow UDF in Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.0) step is set to None, the pool automatically completes AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.0) step and gets removed from the workflow.

Step 4: Demultiplexing (NextSeq 1000/2000 Sequencing v2.0)

This step is semiautomated.

For the demultiplexing information in the Sample Details table to display correctly, the following conditions must be met:

- The DRAGEN version must be earlier than v3.9.3.
- The samples in the run must not repeat.
- The run must be for the single lane flow cell (P1 or P2).

Do not add samples to the Ice Bucket or start or complete the step. The integration completes these actions automatically.

The integration starts the step automatically and demultiplexing data from the GenerateFASTQ secondary analysis is parsed back to Clarity LIMS. Review the data parsed and assign QC, depending on the criteria set, and complete the step.

In the Record Details screen:

1. Review demultiplexing data.
   - Demultiplexing metrics are recorded for the library pool in the Sample Details table. Metrics include columns for # Reads, # Perfect Index Reads, # One Mismatch Index Reads, and # of >= Q30 Bases (PF).
   - The “Demultiplex_stats.csv” file generated by the Dragen onboard or Cloud analysis module is stored under Files section.
2. Assign QC flags to all the individual sample. There are two ways of doing this step.
   a. Manually assign QC flags through the QC column in Sample Details table.
   b. Automatically assign QC flag by running Assign QC flags automation. This option is for scenarios where a huge number of libraries are involved.

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In the Step Details section, the following step UDF fields are visible. (N is the number of criteria. You can use one or more criteria.)

- Criteria N - Source Data Field—Select from preset options eg # Reads.
- Criteria N - Operator—Select from preset options, for example, >= (greater than or equal to).
- Criteria N - Threshold Value—Enter the desired threshold value.

3. After filling up the criteria fields, select Assign QC flags. This selection triggers the automation script, which loops through each library in the pool and apply QC flag base on the criteria set previously.
   This automation also generates an AssignQC Result file under Files section.
4. Select Next Steps.
   On the Assign Next Steps screen, the Next Step field for all samples is prepopulated with Mark protocol as complete.
5. Select Finish Step.

At this point, the whole NextSeq 1000/2000 Integration workflow is fully validated.

Troubleshooting

If an automation trigger does not appear to run its corresponding scripts, see *Troubleshooting Automation* in the Clarity LIMS API and Database documentation.

If the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.0) step starts but does not finish, complete the following steps:

1. Log in to Clarity LIMS using the default user account and use one of the following methods to open the step in Clarity LIMS:
   - Method 1: In Lab View, find the step in the Recent Activities pane.
   - Method 2: Search for the step in Clarity LIMS using reagent cartridge barcode as the search term.
2. On the Record Details screen, locate the Sequencing Log field.
   The multiline text field contains logging information.

If you cannot reach the Record Details screen, or if the Sequencing Log field does not contain enough information to resolve the issue, contact the Clarity LIMS support team. Supply the relevant information from the troubleshooting steps already performed.

NextSeq 1000/2000 Integration v2.1 Release Notes

The following release notes detail the key changes to software components for the Clarity LIMS NextSeq 1000/2000 Integration package since v2.0.

This optional software update is for customers interested in the new features available with this new release.

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If upgrading from a version prior to v2.0, review *NextSeq 1000/2000 Integration v2.0 Release Notes* on page 1754 for a list of features and bug fixes introduced in that version.

Compatibility

- Clarity LIMS v5.3 and later
- NextSeq 1000/2000 Instrument Control Software v1.1 through 1.4
- NGS Extensions Package v5.20.3
- Illumina Preset Protocol (IPP) v2.2.0

New Features

This release of the Clarity LIMS integration package:

- Supports P1 and P3 reagents.
- Supports multiple DRAGEN Analysis versions
  - 3.5.6
  - 3.5.8
  - 3.7.4
  - 3.8.4
- ProjectName is now included in sample sheet v2 generated by the system to support Illumina Connected Analytics integration.

Defect Repairs

- Race condition observed when the final run primary metrics were retrieved from BSSH is now fixed.
- The `configure_sis_gss.sh` script now only update application properties upon success.

Known Issues

- When the disk space is full on instrument, a sequencing run error is triggered. This error causes the generation of an empty `RunParameters.xml`, and the run event is not processed in Clarity LIMS. The occurrence of this error is mitigated by the on instrument prerun checks that assess the available disk space.

Limitations

For the demultiplexing information in the Sample Details table to display correctly, the following conditions must be met:

- The DRAGEN version must be earlier than v3.9.3.
- The samples in the run must not repeat.
- The run must be for the single lane flow cell (P1 or P2).

NextSeq 1000/2000 Integration v2.0 Configuration and User Interaction

The Illumina NextSeq 1000/2000 Integration Package v2.0 supports the integration of Clarity LIMS to Illumina NextSeq 1000/2000 instruments.

This user and configuration guide describes the integration and includes information about preconfigured workflows, protocols, steps, and automations. This guide also lists installed components and provides configuration requirements, rules, and constraints.

For instructions on validating and troubleshooting the Illumina NextSeq 1000/2000 Integration, see *NextSeq 1000/2000 Integration v2.0 Validation and Troubleshooting* on page 1745.

> The configuration provided in this integration has been established to support NextSeq 1000/2000 lab processes. Any configuration changes to protocols or workflows—including renaming protocols, steps, and fields—could break the process.

Overview

The integration includes the following features:

- Preconfigured NextSeq 1000/2000 Sequencing v1.1 workflow that maps to lab protocols and instrument runs.
- Preconfigured protocols:
  - Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.1)
  - Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.1)
  - AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.1)
  - Demultiplexing (NextSeq 1000/2000 Sequencing v1.1)
- Automatic validation of run setup information before sample sheet generation or planned run creation.
- Automated generation of a sample sheet. The sample sheet is used to start the sequencing run on NextSeq 1000/2000 Control Software via Local run mode.
- Automated creation of a planned run on Illumina Connected Analytics (ICA). The planned run created is retrieved by NextSeq 1000/2000 Control Software to start run via Hybrid or Cloud run mode.
- Automated tracking of the NextSeq 1000/2000 sequencing run and parsing of run statistics from BaseSpace Sequence Hub (BSSH) into Clarity LIMS using SIS integration service.
- Automated tracking of the secondary analysis using DRAGEN (up to BCL Convert only) and parsing of demultiplexing metrics from BaseSpace Sequencing Hub (BSSH) into Clarity LIMS using SIS integration service.
- [Optional] Preconfigured Library Prep Validation v2.0.2 workflow used for validation purposes only. The workflow contains a single-step protocol that models the library prep workflow required to produce libraries tagged with index sequences. At the end of the step, these libraries are routed to NextSeq 1000/2000 Sequencing v1.1 workflow. See *NextSeq 1000/2000 Integration v2.0 Validation and Troubleshooting* on page 1745.

Information on these features is provided in the following sections.

Prerequisites and Assumptions

It is assumed that samples entering the NextSeq 1000/2000 Sequencing v1.1 workflow have gone through library preparation and quantification processes. Before they are assigned to the workflow, samples have completed the following actions:

- Samples have been accessioned into Clarity LIMS.
- Samples have been run through QC and library prep.

For details on sample accessioning, see articles in the Samples section of the Clarity LIMS documentation for the appropriate version.

Samples can be assigned to the NextSeq 1000/2000 Sequencing v1.1 workflow automatically (using a routing script) or manually from the Projects and Samples dashboard. See the *Assigning Samples to Workflows* article in the Clarity LIMS documentation.

Workflows, Protocols, and Steps

The Illumina NextSeq 1000/2000 Integration Package v2.0 includes two workflows:

- Library Prep Validation v2.0.2 (optional, but recommended for validation purposes)
- NextSeq 1000/2000 Sequencing v1.1

The following table lists and describes the protocols and steps included in these workflows.

Table 353 Illumina NextSeq 1000/2000 Integration Package v2.0 Protocols and Steps

Protocol Name	Protocol Purpose	Steps Included in Protocol
Library Prep Validation v2.0.2 Workflow		
Library Prep Validation v2.0.2	Included for validation purposes only, this protocol models the library prep steps required to advance samples to the NextSeq 1000/2000 Sequencing v1.1 workflow. The protocol contains a single step—Library Prep Validation v2.0.2. At the end of this step, a routing script sends the samples to the first step of the NextSeq 1000/2000 Sequencing v1.1 workflow. The first step is Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.1).	1. Library Prep Validation v2.0.2
NextSeq 1000/2000 Sequencing v1.1 workflow		

Protocol Name	Protocol Purpose	Steps Included in Protocol
NextSeq 1000/2000 Sequencing v1.1	This protocol models the lab processes of starting a NextSeq 1000/2000 sequencing run. Details of the steps: 1. Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.1) a. Samples are pooled and diluted to the final loading concentration with the help of the Calculate Volume script. 2. Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.1) a. The library pool from step 1 is then ready to be loaded to the NextSeq 1000/2000 reagent cartridge. b. Run and analysis information is validated. c. Sample sheet is generated and/or planned run is created. 3. AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.1) a. This step is a fully automated step that is started and completed automatically after the sequencing run is started and completed on the instrument side. b. All the sequencing run metadata (eg, run configuration, primary run metrics etc.) are recorded automatically. ⚠ Do not add samples to the Ice Bucket or start or complete the step. The integration does this action automatically. c. Samples complete the workflow at this step if GenerateFastQ secondary	1. Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.1) 2. Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.1) 3. AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.1) 4. Demultiplexing (NextSeq 1000/2000 Sequencing v1.1)

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Step Automations

Automation details are provided in the sections following the table.

For details on the validation workflow, see *NextSeq 1000/2000 Integration v2.0 Validation and Troubleshooting* on page 1745.

Table 354 NextSeq 1000/2000 Sequencing v1.1 Workflow Step Automations

Protocol	Step	Automation
NextSeq 1000/2000 Sequencing v1.1	Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.1)	• Calculate Volumes • Set Next Step
	Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.1)	• Set Next Step • Validate Single Input • Validate Reagent Cartridge Barcode • Validate Run Setup and Create Planned Run
	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.1)	• Set Next Step
	Demultiplexing (NextSeq 1000/2000 Sequencing v1.1)	• Assign Demultiplex QC flags • Set Next Step

Terminology

The Illumina NextSeq 1000/2000 Integration Package v2.0 supports Clarity LIMS v5.3 and later.

As of Clarity LIMS v5, the term user-defined field (UDF) has been replaced with custom field in the user interface (the API resource is still called UDF). There are two types of custom fields:

- Master step fields—Configured on master steps. These fields only apply to the master step on which they are configured, and the steps derived from those master steps.
- Global fields—Configured on entities such as submitted sample, derived sample, measurement, etc. These fields apply to the entire Clarity LIMS system.

Step 1: Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.1)

In this step, pooled samples are diluted by the addition of RSB. Manually create a working pool based on the final loading concentration required.

Only create one pool per step for the Calculate Volume automation script to work properly.

Automations

The following automations are configured on the step:

- Calculate Volumes
- Set Next Step
- Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.1) automation configuration

These automations are described in the order in which they are triggered at run time.

Select the Calculate Volumes button on the Record Details screen to trigger this automation automatically, which does the following actions:

- Sets RSB Volume for Pool (µl) field value to 24 for calculation purpose of the 2 nM intermediate library pool.
- Copies the Final Loading Concentration (pM) from step inputs to step outputs.

```
-exp 'step.::RSB Volume for Pool (µl):: = 24; output.::Final Loading
Concentration (pM):: = step.::Final Loading Concentration (pM)::'
```

- Calculate per sample volume required for each library to make the 2 nM intermediate library pool (24ul in volume).

```
-exp 'input.::Per Sample Volume (µl):: = ( 48/ input.::Molarity (nM):: );
step.::RSB Volume for Pool (µl):: -= input.::Per Sample Volume (µl)::'
```

- Calculate the volume required of the 2 nM library pool to be diluted further to the Final Loading Concentration (pM) with the Final Loading Volume (µl).

```
-exp 'step.::Library Pool Volume (µl):: = (step.::Final Loading Concentration
(pM):: * step.::Final Loading Volume (µl)::/2000); output.::RSB Volume (µl):: =
step.::Final Loading Volume (µl):: - step.::Library Pool Volume (µl)::'
```

- Uses the `NextSeq1K2K_Pool1.csv`, `NextSeq1K2K_Pool2.csv`, and `NextSeq1K2K_Pool3.csv` template files to generate a single CSV file containing information about the pool and the samples it contains. The generated file is stored in the Calculation File placeholder, in the Files section, for download.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} \
   script:driver_file_generator \
      -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NextSeq1K2K_
      Pool1.csv \
      -o 1.csv \
   script:driver_file_generator \
      -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NextSeq1K2K_
      Pool2.csv \
```


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```
        -o 2.csv \
    script:driver_file_generator \
        -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NextSeq1K2K_
        Pool3.csv \
        -o 3.csv \
&& cat 1.csv 2.csv 3.csv > {compoundOutputFileLuid0}.csv
```

Automatically triggered on exit of the Record Details screen, this automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol. The next step is Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.1):

```
nextStep = ::ADVANCE::
```

Master Step Fields

There are four fields defined on the Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.1) step.

Table 355 Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.1) Master Step Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Final Loading Concentration (pM)	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 650 • 750 • 1000 • 2000
Final Loading Volume (µl)	Numeric	• Required: Yes • Read Only: No	• Default: 24
Library Pool Volume (µl)	Numeric	• Required: No • Read Only: Yes	Not applicable
RSB Volume for Pool (µl)	Numeric	• Required: No • Read Only: Yes	Not applicable

Global Fields

The following table lists the global fields that are configured to display on the Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.1) step.

Table 356 Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.1) Global Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Final Loading Concentration (pM)	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 225 • 400 • Decimal Places Displayed: 0
RSB Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2

User Interaction

1. On the Queue screen, add samples to the Ice Bucket and select View Ice Bucket.

   PhiX control can be added here if needed.

2. On the Ice Bucket screen, select Begin Work.
3. On the Pooling screen:
   - Create a pool by dragging samples into the Pool Creator.
   - Name the pool or accepts the default name (Pool #1).
   - Select Record Details.
4. On the Record Details screen, the Reagent Lot Tracking section tracks the Resuspension Buffer (RSB) lot information used in the step. You might need to add and activate lots for RSB.
   To add and activate reagent lots:
   a. Navigate to the Consumables configuration screen.
   b. Select the RSB reagent kit and select New Lot.
   c. Enter the lot details and select Save.
   d. Set the Status of Reagent Lot toggle switch to Active.
5. In the Reagent Lot Tracking section, select from the active lots displayed in the drop-down list.
6. The Step Details area contains two required fields:
   - Final Loading Concentration (pM)—The value entered in this field is the recommended final loading concentration depending on the library type. There is a default drop-down list of 650, 750, 1000, and 2000 (custom value is acceptable). For more information, refer to the *NextSeq 1000 and 2000 System Guide*.

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- Final Loading Volume (µl)—The value in this field is the final loading volume of the pool into the reagent cartridge. The field is prepopulated with the configured default value, 24 µl, but can be edited if more volume is required.

7. Select Calculate Volumes. This selection triggers the Calculate Volumes automation. This automation calculates the volume required for each library to form a pool that has the concentration and volume specified in the step details fields.
   The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains volume information for each of the samples and the RSB buffer to add to the pool. Select the file to download it, then open it in Excel.
8. In the Sample Details table, select the pool icon to view details on the pool composition.
   Populates the RSB Volume (UL) and LIMS ID (Container) columns of the Sample Details table.
9. Select Next Steps. This selection triggers the Set Next Step automation. This automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol. The next step in the protocol is Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.1).
10. Select Finish Step.

At the end of this step, the pool of samples automatically advances to Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.1) step.

Step 2: Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.1)

In this step, scan the reagent cartridge barcode into Clarity LIMS, then manually place the working pool into the reagent cartridge for the NextSeq 1000/2000 run. This step validates the run setup and analysis information and generates sample sheet file and/or creates a planned run on ICA, depending on the Run Mode selected.

Automations

The following automations are configured on the step:

- Set Next Step
- Validate Reagent Cartridge Barcode
- Validate Run Setup and Create Planned Run
- Validate Single Input
- Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.1) automation configuration

These automations are described in the order in which they are triggered at run time.

Validate Single Input

Automatically triggered at the beginning of the step, this automation checks that there is only one container input to the step.

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```
script:validateSampleCount -min 1 -max 1
```

Validate Reagent Cartridge Barcode

Automatically triggered on exit of the Placement screen, this automation does the following action:

- Validates the reagent cartridge barcode to make sure it conforms to the barcode mask [A-Za-z]{2}[0-9]{7}-[A-Za-z0-9]{4}:

```
if (!output.container.name.matches( ::[A-Za-z]{2}[0-9]{7}-[A-Za-z0-9]{4}:: ) )
{fail ( ::Invalid Reagent Cartridge Barcode. Please verify and try again.:: ) }
```

Validate Run Setup and Create Planned Run

Automatically triggered when a button on the Record Details screen is selected, this automation does the following actions:

- Validates the parameters entered on the Record Details screen, which are used to set up the run and generate the sample sheet file and/or create the planned run on ICA.
  - Run Name can only contain alphanumeric, dash, underscore, or period characters. Spaces are not permitted.

```
if (!step.::Run Name::.matches(::[a-zA-Z0-9-_.]+::))
{ fail(::Run Name contains prohibited characters. Allowed characters are: a-z,
A-Z, 0-9, -, _, and .::) }
```

- Checks the Index Reads, Index Read 1, and Index Read 2 field values.
  - If Index Reads is No Index, Index Read 1 and Index Read 2 values must be zero (error results if it is otherwise).
  - If Index Reads is Single Index, Index Read 1 value must be greater than zero and Index Read 2 values must be zero (error results if it is otherwise).
  - If Index Reads is Dual Index, Index Read 1 and Index Read 2 values must be greater than zero (error results if it is otherwise).

```
if (step.::Index Reads:: == ::No Index::)
{ if (step.::Index Read 1:: != 0 || step.::Index Read 2:: != 0) { fail(::Index
Read 1 and Index Read 2 must be 0 if the Index Reads is No Index.::) } }
else
{ if (step.::Index Reads:: == ::Single Index::)
{ if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) { fail(::Index
Read 1 must be greater than 0 and Index Read 2 must be 0 if the Index Reads is
Single Index.::) } }
else
{ if (step.::Index Read 1:: == 0 || step.::Index Read 2:: == 0) { fail(::Index
Read 1 and Index Read 2 must be greater than 0 if the Index Reads is Dual
Index.::) } } }
```


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- Checks the Paired End, Read 2 Cycles, and Index Read 2 field values.
  - If Paired End is set to True and Read 2 Cycles value is zero, an error is generated.
  - If Paired End is set to False and Read 2 Cycles or Index Read 2 values are greater than zero, an error is generated.

```
if (step.::Paired End::.toBoolean())
{ if (step.::Read 2 Cycles:: == 0) { fail(::Read 2 Cycles must not be zero if it is Paired End read.::) }}
else
{if (step.::Read 2 Cycles:: != 0 || step.::Index Read 2:: != 0) {fail(::Read 2 Cycles and Index 2 Cycles must be 0 if it is not Paired End Read.:: )}}
```

- Checks the Adapter Sequence Read 1 and Adapter Sequence Read 2 field values.
  - Adapter Sequence Read 1 and Adapter Sequence Read 2 can only contain ACTG characters.

```
if (step.hasValue(::Adapter Sequence Read 1::) && !step.::Adapter Sequence Read 1::.matches(::[ACTG]+::)) { fail(::Adapter Sequence Read 1 contains prohibited characters. Allowed characters are: ACTG::) };
if (step.hasValue(::Adapter Sequence Read 2::) && !step.::Adapter Sequence Read 2::.matches(::[ACTG]+::)) { fail(::Adapter Sequence Read 2 contains prohibited characters. Allowed characters are: ACTG::) }
```

- Check Override Cycles field value.
  - Override Cycles can only contain Y, N, I, U, 0-9, and semicolon characters.

```
if (step.hasValue(::Override Cycles::) && !step.::Override Cycles::.matches(::[YNIU0-9;]+::))
{ fail(::Override Cycles contains prohibited characters. Allowed characters are: Y, N, I, U, 0-9 and ;. Example: N1Y150;I8;I7N1;Y141U10.::) }
```

- Generates the sample sheet and/or create the planned run on ICA.
  - Sample sheet is attached to the step.

```
/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/SIS/SISServices/extensions/automation/nextseq1k2k-automation.jar
script:generate_v2_sample_sheet -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid2} -c {compoundOutputFileLuid0}
```

  - There is backend validation of the sample sheet content to ensure the sample sheet is valid to set up a Local Mode run or ready to be consumed by DRAGEN applications. Refer to *NextSeq 1000/2000 Integration v2.0 Validation and Troubleshooting* on page 1745 for submitted sample name character restriction.

Set Next Step

Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol—AUTOMATED - Sequencing Run NextSeq 1000/2000 Sequencing v1.1.

```
nextStep = ::ADVANCE::
```

Master Step Fields

There are 20 fields defined on the Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.1) step. These fields are required for sample sheet generation and planned run creation on ICA.

Table 357 Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.1) Master Step Fields Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Run Name	Text	• Required: Yes • Read Only: No	Not applicable
Instrument Type	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• NextSeq1000 • NextSeq2000
Run Mode	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• Local • Hybrid • Cloud
Paired End	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• True • False
Read 1 Cycles	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 151 • 101 • 51
Read 2 Cycles	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 151 • 101 • 51
Index Reads	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• No Index • Single Index • Dual Index

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Index Read 1	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 0 • 6 • 8
Index Read 2	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 0 • 6 • 8
Analysis Workflow	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• GenerateFASTQ • None
Adapter Sequence Read 1	Text	• Required: No • Read Only: No	Not applicable
Adapter Sequence Read 2	Text	• Required: No • Read Only: No	Not applicable
Barcode Mismatches Index 1	Numeric Dropdown	• Required: No • Read Only: No • Custom Entries: No	• 0 • 1 • 2
Barcode Mismatches Index 2	Numeric Dropdown	• Required: No • Read Only: No • Custom Entries: No	• 0 • 1 • 2
Override Cycles	Text	• Required: No • Read Only: No	Not applicable
Cloud Run ID	Text	• Required: No • Read Only: Yes	• Hidden - Used in creating planned run in ICA

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Use Custom Index Read 1 Primer	Toggle Switch	• Required: No • Read Only: No	• Hidden Custom primer option is not supported at the moment. Do not enable as it causes integration to break.
Use Custom Index Read 2 Primer	Toggle Switch	• Required: No • Read Only: No	• Hidden Custom primer option is not supported at the moment. Do not enable as it causes integration to break.
Use Custom Read 1 Primer	Toggle Switch	• Required: No • Read Only: No	• Hidden Custom primer option is not supported at the moment. Do not enable as it causes integration to break.

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Use Custom Read 2 Primer	Toggle Switch	• Required: No • Read Only: No	• Hidden Custom primer option is not supported at the moment. Do not enable as it causes integration to break.

User Interaction

1. On the Queue screen, add the sample to the Ice Bucket and select View Ice Bucket.

   Only one sample is allowed.

   On entry to Placement screen, a validation checks if there is only one single input.
2. On the Ice Bucket screen, select Begin Work.
3. On the Placement screen:
   a. Drag the pool into the reagent cartridge in the Placed Samples area.
   b. Enter the barcode for the reagent cartridge by scanning or typing manually in the reagent container field.
   c. Select Record Details.

   On exiting the Placement screen, the Validate Reagent Cartridge Barcode automation checks that the reagent cartridge barcode conforms to the barcode mask [A-Za-z]{2}[0-9]{7}-[A-Za-z0-9]{4}. If not, an error message displays and you are blocked from proceeding.
4. On the Record Details screen, the Reagent Lot Tracking section tracks the NextSeq 1000/2000 Reagent Cartridge used in the step. Select from the active lots displayed in each drop-down list.

   To make reagent lots available for selection in the Reagent Lot Tracking section, configure and activate them. Do this step on the Configuration > Consumables screen.

   The fields displayed on the Run and Analysis Configuration section are used to set up the run and generate the sample sheet file.
   Some of these fields are autopopulated and some must be completed manually:

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- Run Name—Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.
- Instrument Type—Select from preset options: NextSeq 1000 or NextSeq 2000.
- Run Mode—Select from preset options: Local, Hybrid, Cloud. See the *Run Mode Details* section for more information on different run mode.
- Paired End—Select from preset options: True or False.
- Read 1 Cycles—Select from preset options (151, 101, 51) or type a custom value.
- Read 2 Cycles—Select from preset options (151, 101, 51) or type a custom value.
- Index Reads—Select from preset options: No Index, Single Index, Dual Index.
- Index Read 1—Select from preset options (0, 6, 8) or type a custom value.
- Index Read 2—Select from preset options (0, 6, 8) or type a custom value.
- Analysis Workflow—Select from preset options: GenerateFASTQ or None. See the *Analysis Workflow Details* section for more information.
- Adapter Sequence Read 1—Enter the Read 1 adapter sequence of the index adapter kit. Not required if Analysis Workflow is None.
- Adapter Sequence Read 2—Enter the Read 2 adapter sequence of the index adapter kit. Not required if Analysis Workflow is None.
- Barcode Mismatches Index 1—[Optional] Select from preset options: 0, 1, 2. Leave it blank if you are unsure.
- Barcode Mismatches Index 2—[Optional] Select from preset options: 0, 1, 2. Leave it blank if you are unsure.
- Override Cycles—[Optional] String used to specify UMI cycles and mask out cycles of a read (eg, N1Y150;I8;I7N1;Y141U10). Leave it blank if you are unsure.

5. On the Record Details screen, select Validate Run Setup and Create Planned Run.
   The automation script performs the following actions:
   - Validates the parameters entered on the Record Details screen.
   - Creates the planned run and sends it to Illumina Connected Analytics (ICA) if Run Mode is Hybrid or Cloud.
   - Generates the sample sheet and attaches it to the placeholder in the Files area of the Record Details screen for all Run Mode types.

     For Local run mode, download a copy of the sample sheet. It is used to start the sequencing run on the instrument.

6. Select Next Steps.
   On the Assign Next Steps screen, the next step for samples is set to the next step in the workflow—AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.1).

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7. Select Finish Step.

At the end of this step, the pool of samples automatically advances to and is queued for the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.1) step.

Run Mode Details

The run mode applies to all runs and determines how the run and analysis parameters are configured and how to analyze data.

- Local Mode—NextSeq 1000/2000 Integration service generates the sample sheet. Run/Analysis configuration is imported manually into NextSeq 1000/2000 instrument via a sample sheet to start the run. The downstream secondary analysis is done using the onboard DRAGEN module.
- Hybrid Mode—NextSeq 1000/2000 Integration service sends the run/analysis configuration to Illumina Connected Analytics (ICA). Run/Analysis configuration is downloaded to NextSeq 1000/2000 instrument via ICA to start the run. The downstream secondary analysis is done using the onboard DRAGEN module.
- Cloud Mode—NextSeq 1000/2000 Integration service sends the run/analysis configuration to Illumina Connected Analytics (ICA). Run/Analysis configuration is downloaded to NextSeq 1000/2000 instrument via ICA to start the run. The downstream secondary analysis is performed in the cloud.

Analysis Workflow Details

The analysis workflow selection determines if the sample is advanced to the Demultiplexing (NextSeq 1000/2000 Sequencing v1.1) step or completed at the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.1) step.

- GenerateFASTQ—After the sequencing run completes, NextSeq 1000/2000 integration service automatically completes AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.1) step and advances the samples to the Demultiplexing (NextSeq 1000/2000 Sequencing v1.1) step.
- None—None is selected to skip the Demultiplexing (NextSeq 1000/2000 Sequencing v1.1) step. NextSeq 1000/2000 integration service automatically completes AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.1) and removes the samples from the workflow.

Step 3: AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.1)

This step is fully automated.

The integrations start the step automatically and data from the run is parsed back to Clarity LIMS. No user interaction is required. In this step, the pooled samples in the reagent cartridge are sequenced in the NextSeq 1000/2000 instrument.

Do not add samples to the Ice Bucket or start or complete the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.1) step. The integration does this step automatically.

Automations

The following automations are configured on the step:

- Set Next Step
  - The Set Next Step automation is the only automation configured on this step. It automatically sets the samples to advance to the next step—Demultiplexing (NextSeq 1000/2000 Sequencing v1.1).

```
nextStep = ::ADVANCE::
```

Master Step Fields

There are 16 fields defined on the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.1) step. These fields are used to display the run status and sequencing run and analysis configuration parsed from the `RunParameters.xml` file of the sequencing run.

Do not modify the name of any of the master step fields. It causes the integration to break.

See the following table for how some of these step fields map to the fields on the `RunParameters.xml` file.

Table 358 Run Parameters and Corresponding Clarity LIMS Step Fields

Master Step Field	RunParameters.xml Field
Current Cycle	Calculated based on CompletedCycles Field
Current Read	Calculated based on CompletedCycles field against PlannedCycles
Flow Cell ID	FlowCellSerialNumber
Flow Cell Lot Number	FlowCellLotNumber
Instrument Control Software Version	ApplicationVersion
Instrument ID	InstrumentSerialNumber
Output Folder	OutputFolder
Reagent Cartridge ID	CartridgeSerialNumber
Reagent Cartridge Lot Number	CartridgeLotNumber
RTA Version	RtaVersion
Run Name	ExperimentName
Secondary Analysis Workflow	SecondaryAnalysisWorkflow

Global Fields

The following global fields are used to capture the run metrics in Clarity LIMS.

- % Bases >=Q30 R1
- % Bases >=Q30 R2
- % Error Rate R1
- % Error Rate R2
- Yield (Gb) R1
- Yield (Gb) R2
- Reads PF R1
- Reads PF R2
- %PF R1
- %PF R2
- % Aligned R1
- % Aligned R2
- % Phasing R1
- % Phasing R2
- % PrePhasing R1
- % PrePhasing R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- Cluster Density R1
- Cluster Density R2

At the end of this step, the pool of samples undergoes either one of the two following actions:

1. If the Analysis Workflow UDF in Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.1) step is set to GenerateFASTQ, the pool automatically advances to and is queued for the Demultiplexing (NextSeq 1000/2000 Sequencing v1.1) step.
2. If the Analysis Workflow UDF in Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.1) step is set to None, the pool automatically completes the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.1) step and gets removed from the workflow.

Step 4: Demultiplexing (NextSeq 1000/2000 Sequencing v1.1)

This step is a semiautomated step.

The integration starts the step automatically and demultiplexing data from the GenerateFASTQ secondary analysis is parsed back to Clarity LIMS. Then review the demultiplexing result parsed into Clarity LIMS, assign QC flags, and complete the step.

For the demultiplexing information in the Sample Details table to display correctly, the following conditions must be met:

- The DRAGEN version must be earlier than v3.9.3.
- The samples in the run must not repeat.
- The run must be for the single lane flow cell (P1 or P2).

Do not add samples to the Ice Bucket or start or complete the step. The integration completes these actions automatically.

Automations

The following automations are configured on the step:

- Assign Demultiplexing QC flags—Automatically assigns QC flags based on the criteria set on the step fields.
- Set Next Step—Automatically sets the next step to Advance and the samples complete the protocol.

Master Step Fields

There are eight fields defined on the Demultiplexing (NextSeq 1000/2000 Sequencing v1.1) step.

The following table lists the master step fields that are configured on the Demultiplexing (NextSeq 1000/2000 Sequencing v1.1) step.

Table 359 Master Step Field Configuration for Demultiplexing (NextSeq 1000/2000 Sequencing v1.1) Step

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Criteria 1 - Source Data Field	Text Dropdown	• Required: No • Read Only: No • Custom Entries: Yes	• # Reads • # Perfect Index Reads • # One Mismatch Index Reads • # of >= Q30 Bases (PF)

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Criteria 2 - Source Data Field	Text Dropdown	• Required: No • Read Only: No • Custom Entries: Yes	• # Reads • # Perfect Index Reads • # One Mismatch Index Reads • # of >= Q30 Bases (PF)
Criteria 1 - Operator	Text Dropdown	• Required: No • Read Only: No • Custom Entries: Yes	• >= • <= • = • !=
Criteria 2 - Operator	Text Dropdown	• Required: No • Read Only: No • Custom Entries: Yes	• >= • <= • = • !=
Criteria 1 - Threshold Value	Numeric	• Required: No • Read Only: No	• Valid integer value
Criteria 2 - Threshold Value	Numeric	• Required: No • Read Only: No	• Valid integer value
Log	Multiline Text	• Required: No • Read Only: Yes	Not applicable
Reagent Cartridge ID	Text	• Required: No • Read Only: Yes	• Hidden • Used for parsing demultiplexing results

Global Fields

There are four fields defined on the Demultiplexing (NextSeq 1000/2000 Sequencing v1.1) step. which are used to display the demultiplexing result metrics for individual library in the sample pool.

The following table lists the global fields that are configured on the Demultiplexing (NextSeq 1000/2000 Sequencing v1.1) step.

Table 360 Global Field Configuration for Demultiplexing (NextSeq 1000/2000 Sequencing v1.1) Step

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
# of >= Q30 Bases (PF)	Numeric	• Required: No • Read Only: Yes	Not applicable
# One Mismatch Index Reads	Numeric	• Required: No • Read Only: Yes	Not applicable
# Perfect Index Reads	Numeric	• Required: No • Read Only: Yes	Not applicable
# Reads	Numeric	• Required: No • Read Only: Yes	Not applicable

User Interaction

1. On the Record Details screen, complete the following fields to define the QC Passed or QC Failed criteria for the demultiplexing result:
   - Criteria 1 - Source Data Field—Select the first source data field criteria type. Valid options include: # Reads, # Perfect Index Reads, # One Mismatch Index Reads, # of >= Q30 Bases (PF).
   - Criteria 2 - Source Data Field—Select the second source data field criteria type. Valid options include: # Reads, # Perfect Index Reads, # One Mismatch Index Reads, # of >= Q30 Bases (PF).
   - Criteria 1 - Operator—Select the operator that defines the range of values to be included in the first criteria source data field. Valid options include: >=, <=, =, !=.
   - Criteria 2 - Operator—Select the operator that defines the range of values to be included in the second criteria source data field. Valid options include: >=, <=, =, !=.
   - Criteria 1 - Threshold Value—Select a threshold value for the source data field 1.
   - Criteria 2 - Threshold Value—Select a threshold value for source data field 1.
2. On the Record Details screen, select Assign Demultiplex QC flags. The automation script does the following action:
   - Assigns QC flags to each individual library based on the criteria set in the step fields.

     It is optional to use Assign Demultiplex QC flags. You can manually assign QC flag through the QC column in Sample Details table.
3. Select Next Steps.

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On the Assign Next Steps screen, the Next Step field for all samples is prepopulated with Mark protocol as complete.

4. Select Finish Step to complete the workflow.

At the end of this step, the whole protocol is completed. Samples are removed from the workflow.

How the NextSeq 1000/2000 Integration Works

Sample Sheet Generation and Planned Run Creation

On the Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.1) step, when the Validate Run Setup and Create Planned Run automation is triggered, all the run and analysis parameters that were entered in the Run Details screen are sent to Illumina Connected Analytics (ICA). ICA validates the run and analysis configuration and sends an error message to Clarity LIMS if the validation failed. If the validation passes, ICA generates the sample sheet (and sends back to Clarity LIMS) and/or creates a planned run depending on the Run Mode selected.

For Local run mode, only the sample sheet is generated and stored on Clarity LIMS. The sample sheet contains all the run and analysis configuration required to start the run on the instrument.

For Hybrid/Cloud run mode, a planned run is created on ICA. This planned run contains all the run and analysis configuration required to start the run on the instrument. The sample sheet is also generated and stored on Clarity LIMS for reference purpose.

Run Status, Primary Metrics and Analysis Results Parsing and Recording

After the sequencing run is started on the instrument, NextSeq 1000/2000 Control Software notifies BaseSpace Sequence Hub (BSSH) that the sequencing run has been started.

BSSH then does the following actions:

- For Local run mode, create a new run on the BSSH database with the run configuration information received from NextSeq 1000/2000 Control Software.
- For Hybrid/Cloud run mode, create a new run on the BSSH database by copying the run configuration information from the planned run created in ICA, using a special Cloud Run ID received from NextSeq 1000/2000 Control Software.

After the run has been created on BSSH, a run update event message that carries the RunStarted equivalent message is created on BSSH and delivered through ICA and, eventually, to Clarity LIMS SQS queue service. The message is grabbed and processed by SIS integration service.

Based on the reagent cartridge barcode information in the event message (see the following example), SIS looks in the queue of AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.1) step for a container with the same reagent cartridge barcode. When identified, the step is started automatically and the Run Status UDF on Record Details screen is updated to RunStarted. SIS then updates all the other step UDFs (eg, Current Read, Output Folder etc.) by parsing the details from the RunParameters.xml uploaded to BSSH by NextSeq 1000/2000 Control Software.

```
#Example of an run update event
{
    "acl": [
        "wid:61dc14e8-d823-3bd1-bea3-66f9c8388254"
    ],
    "apiUrl":
    "https://api.basespace.illumina.com/v2/runs/r.ZvTfDXsNhUyh2kA5S4Zewg",
    "dateModified": "2020-03-26T09:19:58.5546497Z",
    "flowcellBarcode": "11M11COMC",
    "gdsFolderPath": "/Runs/200326_MP2-15_87_11M11COMC_r.ZvTfDXsNhUyh2kA5S4Zewg",
    "gdsVolumeName": "bssh.61dc14e8d8233bd1bea366f9c8388254",
    "id": "r.ZvTfDXsNhUyh2kA5S4Zewg",
    "instrumentRunId": "200326_MP2-15_87_11M11COMC",
    "name": "200326_MP2-15_87_11M11COMC",
    "reagentBarcode": "EC0001990-EC5",
    "sampleSheetName": "SampleSheet.csv",
    "status": "Running",
    "v1pre3Id": "194424236"
}
```

NextSeq 1000/2000 Control Software continues to notify BSSH of any run status change throughout the course of the sequencing run, and the same run update event delivery process follows.

The following table lists out all the run status and how they are being handled by SIS integration service.

Table 361 Run Status and How SIS Integration Service Handles the Status

Run Status	Shown in Step	Details

RunStarted	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.1)	• Run Status UDF is updated to RunStarted.
		• All other step UDFs are updated based on RunParameters.xml detail obtained from BSSH.
		• Reagent cartridge lot information received from BSSH is compared against the details of the preaccessioned reagent cartridge on Clarity LIMS.
		  • If the expiration date is different, it is updated to the date received from BSSH.
		  • If reagent cartridge is not found, it is created and set to Active.
		  • If reagent cartridge is not in Active status, it is set to Active.
		• Multiline Sequencing Log UDF is updated.

Run Status	Shown in Step	Details
RunCompletedSuccessfully	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.1)	• Run Status UDF is updated to RunCompletedSuccessfully • Current Read and Current Cycle UDFs is updated to the final cycle number and read number based on `RunParameters.xml` detail obtained from BSSH. • Sequencing run result (primary metrics) is downloaded from BSSH and the fields in Sample Details table (eg, % Error Rate R1, % Phasing, etc.) are updated with the values. • Multiline Sequencing Log UDF is updated. • SIS autocompletes the step and routes the samples in the reagent cartridge container to the next step (ie, Demultiplexing (NextSeq 1000/2000 Sequencing v1.1) step if GenerateFastQ is selected as the Analysis Workflow in Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.1) step). Otherwise, the step is completed automatically and the samples are removed from the workflow.

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Run Status	Shown in Step	Details
RunErroredOut	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.1)	• Run Status UDF is updated to RunErroredOut • Current Read and Current Cycle UDFs are updated to the final cycle number and read number that the run stopped based on RunParameters.xml detail obtained from BSSH. • Sequencing run result (primary metrics) is downloaded from BSSH and the fields in Sample Details table (eg, % Error Rate R1, % Phasing, etc.) are updated with the values. • Multiline Sequencing Log UDF is updated. • SIS does not autocomplete the step. Complete the step manually.
RunAbortedByUser	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.1)	• Run Status UDF is updated to RunAbortedByUser • Current Read and Current Cycle UDFs are updated to the final cycle number and read number that was aborted based on RunParameters.xml details obtained from BSSH. • Sequencing run result (primary metrics) is downloaded from BSSH and the fields in Sample Details table (eg, % Error Rate R1, % Phasing, etc.) are updated with the values. • Multiline Sequencing Log UDF is updated. • SIS does not autocomplete the step. Complete the step manually.
AnalysisStarted	Demultiplexing (NextSeq 1000/2000 Sequencing v1.1)	• SIS starts the step automatically for the reagent cartridge container. • Multiline Sequencing Log UDF is updated.

Run Status	Shown in Step	Details
AnalysisCompleted	Demultiplexing (NextSeq 1000/2000 Sequencing v1.1)	• SIS downloads the Demultiplex_ stats.csv file, which contains the demultiplexing result from BSSH. • The file is parsed and details are recorded in the Sample Details table for each library in the library pool. • Multiline Sequencing Log UDF is updated. • SIS does not autocomplete the step. Assign the QC flag to the individual library and manually complete the step.

Step-by-Step Instruction to Start A Sequencing Run On Instrument With Different Run Modes

Local Run Mode

1. On the NextSeq 1000/2000 Control Software interface, select the menu button and select Settings.
2. Select Local Run Setup and Proactive, Run Monitoring and Storage*.
3. [Optional] Enter the default output folder location.
4. Select Save.
5. On the main screen, select Start.
6. Sign in to the BaseSpace Sequence Hub (BSSH) account that was used during NextSeq 1000/2000 Integration package installation setup.
   a. Select the workgroup (if applicable).
   b. Select Next.
7. Select Choose, and a file browser pops up.
8. Browse and select the sample sheet generated during the Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.1) step.
   When selected, the sample sheet has its content validated. After the validation passes, run and analysis configuration is parsed and autopopulated in the next page.
9. Select Review to review the sequencing run settings.
10. Select Load and manually load the reagent cartridge and flow cell.
11. Select Sequence and the pre-run check proceeds.
    After the pre-run check passes, the sequencing run starts automatically.

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Hybrid or Cloud Run Mode

1. On the NextSeq 1000/2000 Control Software interface, select the menu button and select Settings.
2. Select Online Run Setup and Proactive, Run Monitoring and Storage*.
3. [Optional] Enter the default output folder location.
4. Select Save.
5. On the main screen, select Start.
6. Sign into the BaseSpace Sequence Hub (BSSH) account that was used during NextSeq 1000/2000 Integration package installation setup.
   a. Select the workgroup (if applicable).
   b. Select Next.
   Planned runs that were created in the Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.1) step, using Hybrid or Cloud run mode, display as options.
7. Select the run and select Review to review the sequencing run settings.
8. Select Load and manually load the reagent cartridge and flow cell.
9. Select Sequence and the pre-run check proceeds.
   After the pre-run check passes, the sequencing run starts automatically.

Components Installed

The following sections describe the various components that are installed by default as part of this integration. These components include files, properties, reagent categories/label groups, reagent kits, control types, and containers. Information on installed workflows, protocols, steps, and automation points is provided in the *Workflows, Protocols, and Steps* section.

The Illumina NextSeq 1000/2000 Integration v1.1 is distributed as two RPM packages:

- BaseSpaceLIMS-SIS-Core
- BaseSpaceLIMS-SIS-Nextseq1k2k

BaseSpaceLIMS-SIS-Core

BaseSpaceLIMS-SIS-Core is a shared component with all other (future) integrations using ICA.

BaseSpaceLIMS-SIS-Nextseq1k2k, as an extension, contains NextSeq 1000/2000 specific integration logic. This logic can be skipped if customers do not have NextSeq 1000/2000 instruments. These two RPM packages must be installed on the Clarity LIMS server.

The BaseSpaceLIMS-SIS-Core RPM installs the following:

- SIS Core JAR file
- SIS Core Automation JAR file
- Configuration file—`application.properties`

  - There are two `application.properties` files. The configuration file shared by all SIS integrations is located at `/opt/gls/clarity/extensions/SIS/SISServices/conf/application.properties`
- Configuration script—`configure_sis_core-v1.sh`
- Two execution scripts:
  - `sis_core`
  - `run_sis_core`

The base installation folder is /opt/gls/clarity/extensions/SIS

BaseSpaceLIMS-SIS-Nextseq1k2k

BaseSpaceLIMS-SIS-Nextseq1k2k, as an extension, contains NextSeq 1000/2000 specific integration logic. This logic can be skipped if customers do not have NextSeq 1000/2000 instruments. These two RPM packages must be installed on the Clarity LIMS server.

The BaseSpaceLIMS-SIS-Nextseq1k2k RPM installs the following:

- SIS Extension JAR file for NextSeq 1000/2000
- NextSeq 1000/2000 Automation JAR file
- Two NextSeq 1000/2000 configuration files:
  - `/opt/gls/clarity/extensions/SIS/SISServices/conf/NextSeq1k2k/application.properties`
  - `/opt/gls/clarity/extensions/SIS/SISServices/extensions/conf/nextseq1k2k.properties`
- Four configuration scripts:
  - `configure_sis_gss.sh`
  - `configure_sequence_hub_access_token.sh`
  - `configure_sis_remove_gss_subscription.sh`
  - `configure_nextseq1k2k.sh`

The base installation folder is /opt/gls/clarity/extensions/SIS/SISServices/extensions

The NextSeq 1000/2000 integration protocols configuration is delivered through the Illumina Preset Protocols (IPP) v2.1, which requires installation of the NGS Extensions Package (v5.20.2 or later).

- If the NGS Extensions Package is not already installed, or if a version earlier than v5.20.2 is installed, the latest version is installed by default with the NextSeq 1000/2000 integration. See Release Notes for details.
- If NGS Extensions Package v5.20.2 or later is already installed, upgrade is not forced or required.

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SIS Core Configuration File

The `application.properties` configuration file is installed to:
`/opt/gls/clarity/extensions/SIS/SISServices/conf/application.properties`

Do not change any of the fields in the configuration file on your own, as it risks breaking the integration.

It is configured by script and the list of properties include:

- System properties

Property	Description	Default Value
sqsPollingInterval	The interval of the main SIS scheduler job to execute. It is in the unit of seconds.	60
maxNumberOfMessages	The maximum number of messages pulled from SQS in one pull.	10
receiveMessageWaitTimeSeconds	The maximum waiting time in seconds when pulling from SQS.	20
maxMessageHandlingRetryTimes	The maximum of message handling retry times in case of failure.	3
runEvents	The key in the stored failure message JSON file.	runEvents

NextSeq 1000/2000 Configuration File

The `application.properties` configuration file is installed to:
`/opt/gls/clarity/extensions/SIS/SISServices/conf/NextSeq1k2k/application.properties`

Do not change any of the fields in the configuration file on your own, as it risks breaking the integration.

It is configured by script and the list of properties include:

- Script configurable properties

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Property	Description	Default Value
sequenceHubApiUri	The BaseSpace Sequence Hub API URL.	https://api.basespace.illumina.com
icaBaseApiUri	The ICA API URL.	https://use1.platform.illumina.com
icaApiKey	The ICA API KEY that is used to enable user to access to ICA.	Not applicable
workgroupId	The Workgroup ID of the BaseSpace Sequence Hub Login Account. A public user does not need this property.	Not applicable

- Script generated properties
  - The properties icaAccessToken and sequenceHubAccessToken are generated by script but stored in Secret Management Util, rather than in `application.properties`.

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Property	Description
subscriptionId	Subscription ID of subscription that created to subscribe Run Status Change from ICA.
useRunSetUpAuth	The default value is true, which means it internally uses instrument run setup token endpoint for token retrieval.

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Property	Description
icaScopes	The ICA scopes granted to the access token. GSS.SAMPLES.CREATE,GSS.SAMPLES.DELETE,GSS.SAMPLES.READ,GSS.SAMPLES.UPDATE,GSS.LIBRARIES.CREATE,GSS.LIBRARIES.DELETE,GSS.LIBRARIES.READ,GSS.LIBRARIES.UPDATE,GSS.SEQUENCINGRUNS.CREATE,GSS.SEQUENCINGRUNS.DELETE,GSS.SEQUENCINGRUNS.READ,GSS.SEQUENCINGRUNS.UPDATE,GSS.LIBRARYPOOLS.READ,GSS.LIBRARYPREPKITS.READ,GSS.INDEXADAPTERKITS.READ,GSS.ANALYSISVERSIONS.READ,ENS.SUBSCRIPTIONS.MANAGE,BSSH.RUNS.READ
region	The AWS region of the ICA. It is derived from the script configurable property sequenceHubApiUri.

- System properties

Property	Description	Default Value
sqsPollingInterval	The interval of the main SIS scheduler job to execute. It is in the unit of seconds.	60
maxNumberOfMessages	The maximum number of messages pulled from SQS in one pull.	10
receiveMessageWaitTimeSeconds	The maximum waiting time in seconds when pulling from SQS.	20

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Property	Description	Default Value
maxMessageHandlingRetryTimes	The maximum of message handling retry times in case of failure.	3
runEvents	The key in the stored failure message JSON file.	runEvents

NextSeq 1000/2000 Extension Configuration File

The `nextseq1k2k.properties` configuration file is installed to:

```
/opt/gls/clarity/extensions/SIS/SISServices/extensions/conf/nextseq1k2k.properties
```

Do not change any of the fields in the configuration file. It risks breaking the integration.

It is configured by script and the list of properties included:

- Script configurable properties

Property	Description	Default Value
automatedStepNames	The sequencing run step name that is supported by NextSeq1k2k extension. Comma separated.	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.1)
analysisStepNames	The demultiplexing step name that is supported by NextSeq1k2k extension. Comma separated.	Demultiplexing (NextSeq 1000/2000 Sequencing v1.1)
containerType	The supported container type used in NextSeq1k2k workflow step Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.1).	NextSeq 1000/2000 Reagent Cartridge

- Script generated properties

Property	Description	Default Value
reagentBarcodeRegex	The reagent barcode regex that is supported by NextSeq1k2k extension.	[A-Za-z]{2}[0-9]{7}-[A-Za-z0-9]{4}
cloudFastqAvdId	In Cloud run mode, the planned run created and sample sheet generated depends on this value.	avd.042b19e86d5646bdb0520c4faa13f411
localFastqAvdId	In Hybrid run mode, the planned run created and sample sheet generated depends on this value.	avd.d7e7858a351948708fd6c15e7298d7db
analysisAppApplicationFamilySlug	In Cloud run mode, it is used to retrieve the demultiplexing stats data from BaseSpace Sequence Hub	illumina-inc.basespace-dragen-analysis
maxWaitingTimeInSeconds	The maximum of waiting time in seconds to retrieve RunParameter.xml from BaseSpace Sequence Hub.	180*60 (180 mins)

Consumables Installed

Reagent Categories/Label Groups

- TruSeq HT Adapters v2 (D7-D5)

Reagent Kits

- Resuspension Buffer (RSB)
- NextSeq 1000/2000 Reagent Cartridge

Container Types

- Tube
- NextSeq 1000/2000 Reagent Cartridge

Control Types

- PhiX v3

This integration supports:

- NextSeq 1000/2000 Reagent Cartridge with barcode provided in the format [A-Za-z]{2}[0-9]{7}-[A-Za-z0-9]{4}
  - Example: EC1234567-EC03

Rules and Constraints

- The workflow configuration contains several validation checks. To make sure that the calculations work properly, it is important that you do not disable any of this validation logic. The validation checks determine:
  - Which samples, and how many, can enter each step together.
  - Which samples, and how many, can be pooled together.
- The NextSeq 1000/2000 reagent cartridge barcode must be unique. There should not be multiple NextSeq 1000/2000 reagent cartridge containers in the system with the same name.
- Reagent labels (indexes) must be unique.
- One library pool can only contain one library or control with no label (index).
- Do not manually start or complete the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.1) step. This step is a fully automated step, and the SIS integration service does not update samples correctly if they have been manually started.
- Do not manually start the Demultiplexing (NextSeq 1000/2000 Sequencing v1.1) step. This step is semiautomated, and the SIS integration service does not update the demultiplexing result correctly if they have been manually started.

- For the automated run to start successfully, select Validate Run Setup and Create Planned Run in the Load to Reagent Cartridge step.

NextSeq 1000/2000 Integration v2.0 Validation and Troubleshooting

This section explains how to validate the installation of the Illumina NextSeq 1000/2000 Integration Package v2.0.

The validation process involves:

- Running samples through the Library Prep Validation v2.0.2 workflow.
  - The workflow contains a single-step protocol that models the library prep workflow required to produce libraries tagged with index sequences. At the end of the step, these libraries are routed to NextSeq 1000/2000 Sequencing v1.1 workflow.
- Running the libraries through the NextSeq 1000/2000 Sequencing v1.1 workflow. This process validates the following information:
  - Successful sequential step advancement of samples in the following steps:
    1. Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.1)
    2. Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.1)
    3. AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.1)
    4. [Optional] Demultiplexing (NextSeq 1000/2000 Sequencing v1.1)
  - Automatic validation of run setup information before sample sheet generation or planned run creation.
  - Automated generation of a sample sheet. The sample sheet is used to start the sequencing run on NextSeq 1000/2000 Control Software via Local run mode.
  - Automated creation of a planned run on Illumina Connected Analytics (ICA). The planned run created is retrieved by NextSeq 1000/2000 Control Software to start run via Hybrid or Cloud run mode.
  - Automated tracking of the NextSeq 1000/2000 sequencing run and parsing of run statistics from BaseSpace Sequence Hub (BSSH) into Clarity LIMS using SIS Integration Service.
  - Automated tracking of the secondary analysis using DRAGEN (up to BCL Convert only) and parsing of demultiplexing metrics from BaseSpace Sequence Hub (BSSH) into Clarity LIMS using SIS integration service.

Before running the validation steps below, these steps assume that the NextSeq 1000/2000 Sequencing v1.1 Integration Package is installed and you have imported the default Clarity LIMS configuration.

For information on how the integration works, refer to *NextSeq 1000/2000 Integration v2.0 Configuration and User Interaction* on page 1708.

Activate Workflow, Create Project, Add and Assign Samples

The following steps set up Clarity LIMS in preparation for running samples through the Library Prep Validation v2.0.2 and NextSeq 1000/2000 Sequencing v1.1 workflows.

1. In the Configuration tab of Clarity LIMS under Workflows, activate both the Library Prep Validation v2.0.2 and NextSeq 1000/2000 Sequencing v1.1 workflows.
2. On the Projects and Samples screen, create a project and add samples to it.
3. Assign the samples to the Library Prep Validation v2.0.2 workflow.

Library Prep Protocol: Library Prep Validation v2.0.2

This single-step protocol models the library prep required to produce libraries tagged with index sequences that are ready for the NextSeq 1000/2000 Sequencing v1.1 workflow.

Run Library Prep Validation v2.0.2 Step

1. In Lab View, locate the Library Prep Validation v2.0.2 protocol. The samples are queued for the Library Prep Validation v2.0.2 step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select the label groups to use.
   - TruSeq HT Adapters v2 (D7-D5) is the default label group delivered by Library Prep Validation v2.0.2 protocol.
   - For other reagent labels (indexes), manually create them in the Configuration screen under Consumables → Labels.

   For more information on how to create reagent label group, refer Clarity LIMS documentation.
4. Select Begin Work.
5. On the Placement screen:
   a. In the Placed Samples area in the Container field, scan or enter the barcode of the destination container.
   b. Select the samples from the Samples to be Placed area, then drag them over to the container wells on the right.
   c. Select Add Labels.
6. On the Add Labels screen, the Labels to Add list is populated with labels from the TruSeq HT Adapters v2 (D7-D5) label group. This label group is the only one configured in the out-of-the-box integration. It is used by default in this step.
   a. Select labels on the left, and drag them over to the container on the right to assign them to placed samples.
   b. Select Record Details.
7. On the Record Details screen:

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a. In the Sample Details table, enter values for Molarity (nM) for all the individual sample. Type values individually or use the copy/paste functionality to enter multiple values from a spreadsheet.
   - Molarity (nM) values are used in Calculate Volume automation script in Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.1) step.

b. The Sequencing Instrument column indicates the instrument to be used for the run. Select NextSeq 1000/2000.

c. Populate the fields in this column using one of the following methods:
   - To populate fields individually for each sample, select from the drop-down list in each row.
   - To populate fields all at one time, use the Copy and Paste buttons to enter multiple values from a spreadsheet (see the following note).

Alternatively, select inside the table and press Ctrl + A to select all rows. At the top of the table, select Sequencing Instrument, then select NextSeq 1000/2000 from the adjacent drop-down list. Select Apply.

> If you are working with many samples, you can use the copy/paste functionality to enter multiple values from a spreadsheet.
>
> a. Select the Copy button to copy the sample information from Clarity LIMS to the clipboard.
> b. Open a blank Excel document and paste the contents of the clipboard into the spreadsheet.
> c. In the spreadsheet, enter the Molarity (nM) and Sequencing Instrument values. Select the entire spreadsheet, including the headers, (Ctrl + A) and copy (Ctrl + C) the information to the clipboard.
> d. In Clarity LIMS, select the Paste button. Paste the contents of the clipboard (Ctrl + V) into the Update Table window and then select Update to paste into the Sample Details table.

8. Select Next Steps.
   This selection triggers the Next Steps automation, which sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step successfully.
9. On the Assign Next Steps screen in the Samples table, the Next Step for all samples is prepopulated with Remove from workflow.

   > Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.

10. Select Finish Step.

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On exit from the step, the Routing Script automation is triggered. This automation assigns samples to the first step of the NextSeq 1000/2000 Sequencing v1.1 workflow, which is Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.1) step.

NextSeq 1000/2000 Sequencing v1.1 Protocol

Step 1: Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.1)

1. In Lab View, locate the NextSeq 1000/2000 Sequencing v1.1 protocol. The samples are queued for the Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.1) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Pooling screen:
   a. Create a pool by dragging samples into the Pool Creator.
   b. Type a name for the pool or accept the default name (Pool #1).
   c. Select Record Details.
5. On the Record Details screen, the Reagent Lot Tracking section tracks the Resuspension Buffer (RSB) lot information used in the step. You might need to add and activate lots for RSB.
   To add and activate reagent lots:
   a. In a new browser tab, open an additional instance of Clarity LIMS.
   b. Navigate to the Consumables configuration screen.
   c. Select the RSB reagent kit and select New Lot.
   d. Enter the lot details and select Save.
   e. Set the Status of Reagent Lot toggle switch to Active.
6. In the Reagent Lot Tracking section, select from the active lots displayed in the drop-down list.
7. The Step Details area contains two required fields:
   - Final Loading Concentration (pM)—The value entered in this field is the recommended final loading concentration from NextSeq 1000/2000 System Guide depending on the library type. There is a default drop-down list of 650, 750, 1000 and 2000. A custom value is acceptable.
   - Final Loading Volume (µl)—The value in this field is the final loading volume of the pool into the reagent cartridge. The field is prepopulated with the configured default value, 24 µl, which is recommended by Illumina NextSeq 1000/2000 Instrument System Guide. However, it can be edited if more volume is necessary.
8. Select Calculate Volumes. This selection triggers the Calculate Volumes automation. This automation calculates the volume required for each library to form a pool that has the concentration and volume specified in the step details fields.

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The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains volume information of each of the samples and RSB buffer to add to the pool. Select the file to download it, then open it in Excel.

9. In the Sample Details table, select the pool icon to view details on the pool composition.
10. Select Next Steps. This selection triggers the Set Next Step automation, which sets the next step for samples to ADVANCE, advancing them to the next step in the protocol. The next step is Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.1).
    On the Assign Next Steps screen, the next step for samples is already set to the next step in the workflow. The next step is Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.1).
11. Select Finish Step.

At the end of this step, the pool of samples automatically advances to Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.1) step.

Step 2: Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.1)

1. In Lab View, locate the NextSeq 1000/2000 Sequencing v1.1 protocol. The pool of samples is queued for the Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.1) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
   The Validate Single Input automation is triggered. This automation checks that there is only one container input to the step.
4. On the Placement screen:
   a. Drag the pool into the NextSeq 1000/2000 Reagent Cartridge in the Placed Samples area.
   b. Scan or type the barcode of the reagent cartridge into the NextSeq 1000/2000 Reagent Cartridge field.
   c. Select Record Details.
   On exit of the Placement screen, the Validate Reagent Cartridge Barcode automation checks that the reagent cartridge barcode conforms to the barcode mask [A-Za-z]{2}[0-9]{7}-[A-Za-z0-9]{4}. If not, an error message displays.
5. On the Record Details screen, the Reagent LotTracking section tracks the NextSeq 1000/2000 Reagent Cartridge lot information used in the step. Follow the steps in Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.1) step if you need to add a new lot.
6. In the Reagent Lot Tracking section, select from the active lots displayed in the drop-down list.
7. The fields displayed on the Record Details screen are used to create planned run and generate the sample sheet file.
   - Run Name—Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.
   - Instrument Type—Select from preset options: NextSeq 1000, NextSeq 2000.

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- Run Mode—Select from preset options: Local, Hybrid, Cloud. See *Run Mode Details* for more information on different run mode.
- Paired End—Select from preset options: True or False.
- Read 1 Cycles—Select from preset options (151, 101, 51) or type a custom value.
- Read 2 Cycles—Select from preset options (151, 101, 51) or type a custom value.
- Index Reads—Select from preset options: No Index, Single Index, Dual Index.
- Index Read 1—Select from preset options (0, 6, 8) or type a custom value.
- Index Read 2—Select from preset options (0, 6, 8) or type a custom value.
- Analysis Workflow—Select from preset options: GenerateFASTQ or None. See *Analysis Workflow Details* for more information.
- Adapter Sequence Read 1—[Optional] Enter the Read 1 adapter sequence of the index adapter kit.
- Adapter Sequence Read 2—[Optional] Enter the Read 2 adapter sequence of the index adapter kit.
- Barcode Mismatches Index 1—[Optional] Select from preset options: 0, 1, 2. Leave it blank if you are unsure.
- Barcode Mismatches Index 2—[Optional] Select from preset options: 0, 1, 2. Leave it blank if you are unsure.
- Override Cycles—[Optional] String used to specify UMI cycles and mask out cycles of a read, eg, N1Y150;I8;I7N1;Y141U10. Leave it blank if you are unsure.

8. On the Record Details screen, select Validate Run Setup and Create Planned Run. This selection triggers the automation script, which does the following actions:
   - Validates the parameters entered on the Record Details screen.
   - Create the planned run and send it to Illumina Connected Analytics (ICA) if Run Mode is Hybrid or Cloud.
   - Generates the sample sheet and attaches it to the placeholder in the Files area of the Record Details screen for all Run Mode types.
9. Select Next Steps.
   On the Assign Next Steps screen, the next step for samples is set to the next step in the workflow. The next step is AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.1).
10. Select Finish Step.

At the end of this step, the pool of samples automatically advances to and is queued for the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.1) step. The sequencing run is ready to be started. For details on how to start the sequencing run for different run modes, refer to *NextSeq 1000/2000 Integration v2.0 Configuration and User Interaction* on page 1708.

Run Mode Details

The run mode applies to all runs and determines how the run and analysis parameters are configured and how to analyze data.

- Local Mode—NextSeq 1000/2000 Integration service generates the sample sheet. Run/Analysis configuration is imported manually into NextSeq 1000/2000 instrument via a sample sheet to start the run. The downstream secondary analysis is done using the onboard DRAGEN module.
- Hybrid Mode—NextSeq 1000/2000 Integration service sends the run/analysis configuration to Illumina Connected Analytics Platform (ICA). Run/Analysis configuration is downloaded to NextSeq 1000/2000 instrument via ICA to start the run. The downstream secondary analysis is done using the onboard DRAGEN module.
- Cloud Mode—NextSeq 1000/2000 Integration service sends the run/analysis configuration to Illumina Connected Analytics (ICA). Run/Analysis configuration is downloaded to NextSeq 1000/2000 instrument via ICA to start the run. The downstream secondary analysis is performed in the cloud.

Analysis Workflow Details

The analysis workflow selection determines if the sample is advanced to Demultiplexing (NextSeq 1000/2000 Sequencing v1.1) step or completed at AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.1) step.

- GenerateFASTQ—After the sequencing run completes, NextSeq 1000/2000 integration service automatically completes the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.1) step. Samples are advanced to the Demultiplexing (NextSeq 1000/2000 Sequencing v1.1) step.
- None—None is selected to skip Demultiplexing (NextSeq 1000/2000 Sequencing v1.1) step. NextSeq 1000/2000 integration service automatically completes the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.1) and removes the samples from the workflow.

Step 3: AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.1) Step



Do not add samples to the Ice Bucket or start or complete the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.1) step. The integration does this action automatically.

This step is fully automated.

The integration starts the step automatically and data from the run is parsed back to Clarity LIMS. No user interaction is required. However, you can open and review the various stages of the step in Clarity LIMS. Do not perform any action when reviewing the data.

Read summary metrics are recorded for the library pool in the Step Details section and the Sample Details table.

Values are populated in the following step UDFs/master step fields:

Run Name	Run Status	Output Folder
Current Read	Current Cycle	Secondary Analysis Workflow
Flow Cell ID	Flow Cell Lot Number	Reagent Cartridge ID
Reagent Cartridge Lot Number	Instrument Platform	Instrument Type
Instrument ID	Instrument Control Software Version	RTA Version
Sequencing Log		

The summary metrics (per run level) populate in the following UDFs/global fields.

- % Bases >=Q30 R1
- % Bases >=Q30 R2
- % Error Rate R1
- % Error Rate R2
- Yield (Gb) R1
- Yield (Gb) R2
- Reads PF R1
- Reads PF R2
- %PF R1
- %PF R2
- % Aligned R1
- % Aligned R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- Cluster Density R1
- Cluster Density R2

At the end of this step, the pool of samples undergoes either one of the following two actions:

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1. If the Analysis Workflow UDF in Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.1) step is set to GenerateFASTQ, the pool automatically advances to and is queued for the Demultiplexing (NextSeq 1000/2000 Sequencing v1.1) step.
2. If the Analysis Workflow UDF in Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.1) step is set to None, the pool automatically completes AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.1) step and gets removed from the workflow.

Step 4: Demultiplexing (NextSeq 1000/2000 Sequencing v1.1)

This step is semiautomated.

For the demultiplexing information in the Sample Details table to display correctly, the following conditions must be met:

- The DRAGEN version must be earlier than v3.9.3.
- The samples in the run must not repeat.
- The run must be for the single lane flow cell (P1 or P2).

Do not add samples to the Ice Bucket or start or complete the step. The integration completes these actions automatically.

The integration starts the step automatically and demultiplexing data from the GenerateFASTQ secondary analysis is parsed back to Clarity LIMS. Review the data parsed and assign QC, depending on the criteria set, and complete the step.

In the Record Details screen:

1. Review demultiplexing data.
   - Demultiplexing metrics are recorded for the library pool in the Sample Details table. Metrics include columns for # Reads, # Perfect Index Reads, # One Mismatch Index Reads, and # of >= Q30 Bases (PF).
   - The “Demultiplex_stats.csv” file generated by the Dragen onboard or Cloud analysis module is stored under Files section.
2. Assign QC flags to all the individual sample. There are two ways of doing this step.
   a. Manually assign QC flags through the QC column in Sample Details table.
   b. Automatically assign QC flag by running Assign QC flags automation. This option is for scenarios where a huge number of libraries are involved.

   In the Step Details section, the following step UDF fields are visible. (N is the number of criteria. You can use one or more criteria.)
   - Criteria N - Source Data Field—Select from preset options eg # Reads.
   - Criteria N - Operator—Select from preset options, for example, >= (greater than or equal to).

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- Criteria N - Threshold Value—Enter the desired threshold value.

3. After filling up the criteria fields, select Assign QC flags. This selection triggers the automation script, which loops through each library in the pool and apply QC flag base on the criteria set previously.
   This automation also generates an AssignQC Result file under Files section.
4. Select Next Steps.
   On the Assign Next Steps screen, the Next Step field for all samples is prepopulated with Mark protocol as complete.
5. Select Finish Step.

At this point, the whole NextSeq 1000/2000 Integration workflow is fully validated.

Troubleshooting

If an automation trigger does not appear to run its corresponding scripts, see *Troubleshooting Automation* in the Clarity LIMS API documentation.

If the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.1) step starts but does not finish, complete the following steps:

1. Log in to Clarity LIMS using the default user account and use one of the following methods to open the step in Clarity LIMS:
   - Method 1: In Lab View, find the step in the Recent Activities pane.
   - Method 2: Search for the step in Clarity LIMS using reagent cartridge barcode as the search term.
2. On the Record Details screen, locate the Sequencing Log field.
   The multiline text field contains logging information.

If you cannot reach the Record Details screen, or if the Sequencing Log field does not contain enough information to resolve the issue, contact the Clarity LIMS support team. Supply the relevant information from the troubleshooting steps already performed.

NextSeq 1000/2000 Integration v2.0 Release Notes

The following release notes detail the key changes to software components for the Clarity LIMS NextSeq 1000/2000 Integration package v2.0.

Compatibility

- Clarity LIMS Hosted v5.3 through 5.4
- NGS Extensions Package v5.20 or later
- Illumina Preset Protocol (IPP) v2.1.0 or later
- NextSeq 1000/2000 Instrument Control Software v1.1
- DRAGEN v3.5.8

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New Features

This release of the Clarity LIMS integration package:

- Supports the security improvements made to the management of passwords in Clarity LIMS v5.4.
- Decouples the SIS CORE from the integration package extension. Each is available for download independently.
- References ICA instead of IAP.
- Supports AWS SDK 2.6.15 to mitigate the risk of its dependency to `Jackson-databind.jar`.

Known Issues

- None

Limitations

For the demultiplexing information in the Sample Details table to display correctly, the following conditions must be met:

- The DRAGEN version must be earlier than v3.9.3.
- The samples in the run must not repeat.
- The run must be for the single lane flow cell (P1 or P2).

NextSeq 1000/2000 Integration v1.0 Configuration and User Interaction

The Illumina NextSeq 1000/2000 Integration Package v1.0 supports the integration of Clarity LIMS to Illumina NextSeq 1000/2000 instruments.

This user and configuration guide describes the integration and includes information about preconfigured workflows, protocols, steps, and automations. This guide also lists installed components and provides configuration requirements, rules, and constraints.

For instructions on validating and troubleshooting the Illumina NextSeq 1000/2000 Integration, see the *NextSeq 1000/2000 Integration v1.0 Validation and Troubleshooting Guide*.

> The configuration provided in this integration has been established to support NextSeq 1000/2000 lab processes. Any configuration changes to protocols or workflows—including renaming protocols, steps, and fields—could break the process.

The integration includes the following features:

- Preconfigured NextSeq 1000/2000 Sequencing v1.0 workflow that maps to lab protocols and instrument runs.
- Preconfigured protocols:
  - Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.0)
  - Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.0)
  - AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.0)
  - Demultiplexing (NextSeq 1000/2000 Sequencing v1.0)

- Automatic validation of run setup information before sample sheet generation or planned run creation.
- Automated generation of a sample sheet. The sample sheet is used to start the sequencing run on NextSeq 1000/2000 Control Software via Local run mode.
- Automated creation of a planned run on Illumina Analytics Platform (IAP). The planned run created is retrieved by NextSeq 1000/2000 Control Software to start run via Hybrid or Cloud run mode.
- Automated tracking of the NextSeq 1000/2000 sequencing run and parsing of run statistics from BaseSpace Sequence Hub (BSSH) into Clarity LIMS using SIS integration service.
- [Optional] Preconfigured Library Prep Validation v2.0 workflow used for validation purposes only. The workflow contains a single-step protocol that models the library prep workflow required to produce libraries tagged with index sequences. At the end of the step, these libraries are routed to NextSeq 1000/2000 Sequencing v1.0 workflow. See the *NextSeq 1000/2000 Integration v1.0 Validation and Troubleshooting Guide*.

Information on these features is provided in the following sections.

Prerequisites and Assumptions

It is assumed that samples entering the NextSeq 1000/2000 v1.0 workflow have gone through library preparation and quantification processes. Before they are assigned to the workflow, samples have completed the following actions:

- Samples have been accessioned into Clarity LIMS.
- Samples have been run through QC and library prep.

For details on sample accessioning, see articles in the Samples section of the Clarity LIMS documentation for the appropriate version.

Samples can be assigned to the NextSeq 1000/2000 Sequencing v1.0 workflow automatically (using a routing script) or manually from the Projects and Samples dashboard. See the *Assigning Samples to Workflows* article in the Clarity LIMS documentation.

Workflows, Protocols, and Steps

The Illumina NextSeq 1000/2000 Integration Package v1.0 includes two workflows:

- NextSeq 1000/2000 Sequencing v1.0
- Library Prep Validation v2.0 (optional, but recommended for validation purposes)

Table 1 lists and describes the protocols and steps included in these workflows.

Table 362 Table 1: Illumina NextSeq 1000/2000 Integration Package v1.0 Protocols and Steps

Protocol Name	Protocol Purpose	Steps Included in Protocol
Library Prep Validation v2.0 Workflow		
Library Prep Validation v2.0	Included for validation purposes only, this protocol models the library prep steps required to advance samples to the NextSeq 1000/2000 Sequencing v1.0 workflow. The protocol contains a single step—Library Prep Validation v2.0. At the end of this step, a routing script sends the samples to the first step of the NextSeq 1000/2000 Sequencing v1.0 workflow. The first step is Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.0).	1. Library Prep Validation v2.0
NextSeq 1000/2000 Sequencing v1.0 workflow		

Protocol Name	Protocol Purpose	Steps Included in Protocol
NextSeq 1000/2000 Sequencing v1.0	This protocol models the lab processes of starting a NextSeq 1000/2000 sequencing run. Details of the steps: 1. Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.0) a. Samples are pooled and diluted to the final loading concentration with the help of the Calculate Volume script. 2. Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.0) a. The library pool from step 1 is then ready to be loaded to the NextSeq 1000/2000 reagent cartridge. b. Run and analysis information is validated. c. Sample sheet is generated and/or planned run is created. 3. AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.0) a. This step is a fully automated step that is started and completed automatically after the sequencing run is started and completed on the instrument side. b. All the sequencing run metadata (eg, run configuration, primary run metrics etc.) are recorded automatically. ⚠ Do not add samples to the Ice Bucket or start or complete the step. The integration does this action automatically. c. Samples complete the workflow at this step if GenerateFastQ secondary	1. Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.0) 2. Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.0) 3. AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.0) 4. Demultiplexing (NextSeq 1000/2000 Sequencing v1.0)

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Validation Workflow

The Library Prep Validation v2.0 workflow allows for validation of the system after installation is complete. For details, see the*NextSeq 1000/2000 Sequencing v1.0 - Validation and Troubleshooting Guide*.

Step Automations

Table 2 lists the step automations included in the NextSeq 1000/2000 Sequencing v1.0 workflow. Automation details are provided in the sections following the table.

For details on the validation workflow, see the *NextSeq 1000/2000 Integration v1.0 Validation and Troubleshooting Guide*.

Table 363 Table 2: NextSeq 1000/2000 Sequencing v1.0 Workflow Step Automations

Protocol	Step	Automation
NextSeq 1000/2000 Sequencing v1.0	Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.0)	• Calculate Volumes • Set Next Step
	Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.0)	• Set Next Step • Validate Single Input • Validate Reagent Cartridge Barcode • Validate Run Setup and Create Planned Run
	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.0)	• Set Next Step
	Demultiplexing (NextSeq 1000/2000 Sequencing v1.0)	• Assign Demultiplex QC flags • Set Next Step

Configuration Details

The following sections describe the configuration installed with the Illumina NextSeq 1000/2000 Integration.

The Illumina NextSeq 1000/2000 Integration Package v1.0 supports Clarity LIMS v5.1 and later.

As of Clarity LIMS v5, the term user-defined field (UDF) has been replaced with custom field in the user interface (the API resource is still called UDF). There are two types of custom fields:

- Master step fields—Configured on master steps. These fields only apply to the master step on which they are configured, and the steps derived from those master steps.
- Global fields—Configured on entities such as submitted sample, derived sample, measurement, etc. These fields apply to the entire Clarity LIMS system.

NextSeq 1000/2000 v1.0 Protocol

Samples are routed to this protocol after they have gone through Library Prep Validation v2.0 (or any other library prep workflow). Samples are pooled, diluted to the final loading concentration, and added to a reagent cartridge in preparation for the NextSeq run.

This protocol contains four steps:

1. Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.0)
2. Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.0)
3. AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.0)
4. Demultiplexing (NextSeq 1000/2000 Sequencing v1.0)

Step 1: Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.0)

In this step, pooled samples are diluted by the addition of RSB. Manually create a working pool based on the final loading concentration required.

Only create one pool per step for the Calculate Volume automation script to work properly.

Automations

The following automations are configured on the step:

- Calculate Volumes
- Set Next Step
- Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.0) automation configuration

These automations are described in the order in which they are triggered at run time.

Select the Calculate Volumes button on the Record Details screen to trigger this automation automatically, which does the following actions:

- Sets RSB Volume for Pool (µl) field value to 24 for calculation purpose of the 2 nM intermediate library pool.
- Copies the Final Loading Concentration (pM) from step inputs to step outputs.

```
-exp 'step.::RSB Volume for Pool (µl):: = 24; output.::Final Loading
Concentration (pM):: = step.::Final Loading Concentration (pM)::'
```


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- Calculate per sample volume required for each library to make the 2 nM intermediate library pool (24ul in volume).

```
-exp 'input.::Per Sample Volume (µl):: = ( 48/ input.::Molarity (nM):: );
step.::RSB Volume for Pool (µl):: -= input.::Per Sample Volume (µl)::'
```

- Calculate the volume required of the 2 nM library pool to be diluted further to the Final Loading Concentration (pM) with the Final Loading Volume (µl).

```
-exp 'step.::Library Pool Volume (µl):: = (step.::Final Loading Concentration
(pM):: * step.::Final Loading Volume (µl)::/2000); output.::RSB Volume (µl):: =
step.::Final Loading Volume (µl):: - step.::Library Pool Volume (µl)::'
```

- Uses the `NextSeq1K2K_Pool1.csv`, `NextSeq1K2K_Pool2.csv`, and `NextSeq1K2K_Pool3.csv` template files to generate a single CSV file containing information about the pool and the samples it contains. The generated file is stored in the Calculation File placeholder, in the Files section, for download.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} -l {compoundOutputFileLuid1} \
   script:driver_file_generator \
      -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NextSeq1K2K_
      Pool1.csv \
      -o 1.csv \
   script:driver_file_generator \
      -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NextSeq1K2K_
      Pool2.csv \
      -o 2.csv \
   script:driver_file_generator \
      -t /opt/gls/clarity/extensions/conf/driverfiletemplates/NextSeq1K2K_
      Pool3.csv \
      -o 3.csv \
&& cat 1.csv 2.csv 3.csv > {compoundOutputFileLuid0}.csv
```

Automatically triggered on exit of the Record Details screen, this automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol. The next step is Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.0):

```
nextStep = ::ADVANCE::
```

Master Step Fields

There are four fields defined on the Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.0) step.

These fields are shown in the Configuration area (Custom Fields > Master Step Fields tab). See Table 3 for the field configuration details.

Table 364 Table 3: Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.0) Master Step Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Final Loading Concentration (pM)	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 650 • 750 • 1000 • 2000
Final Loading Volume (µl)	Numeric	• Required: Yes • Read Only: No	• Default: 24
Library Pool Volume (µl)	Numeric	• Required: No • Read Only: Yes	Not applicable
RSB Volume for Pool (µl)	Numeric	• Required: No • Read Only: Yes	Not applicable

Global Fields

Table 4 lists the global fields that are configured to display on the Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.0) step.

Table 365 Table 4: Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.0) Global Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Final Loading Concentration (pM)	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 225 • 400 • Decimal Places Displayed: 0
RSB Volume (µl)	Numeric	• Required: No • Read Only: Yes	• Decimal Places Displayed: 2

User Interaction

1. On the Queue screen, add samples to the Ice Bucket and select View Ice Bucket.

   PhiX control can be added here if needed.

2. On the Ice Bucket screen, select Begin Work.
3. On the Pooling screen:
    - Create a pool by dragging samples into the Pool Creator.
    - Name the pool or accepts the default name (Pool #1).
    - Select Record Details.
4. On the Record Details screen, the Reagent Lot Tracking section tracks the Resuspension Buffer (RSB) lot information used in the step. You might need to add and activate lots for RSB.
   To add and activate reagent lots:
    a. Navigate to the Consumables configuration screen.
    b. Select the RSB reagent kit and select New Lot.
    c. Enter the lot details and select Save.
    d. Set the Status of Reagent Lot toggle switch to Active.
5. In the Reagent Lot Tracking section, select from the active lots displayed in the drop-down list.
6. The Step Details area contains two required fields:
    - Final Loading Concentration (pM)—The value entered in this field is the recommended final loading concentration depending on the library type. There is a default drop-down list of 650, 750, 1000, and 2000 (custom value is acceptable).
    - Final Loading Volume (µl)—The value in this field is the final loading volume of the pool into the reagent cartridge. The field is prepopulated with the configured default value, 24 µl, but can be edited if more volume is required.
7. Select Calculate Volumes. This selection triggers the Calculate Volumes automation. This automation calculates the volume required for each library to form a pool that has the concentration and volume specified in the step details fields.
   The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains volume information for each of the samples and the RSB buffer to add to the pool. Select the file to download it, then open it in Excel.
8. In the Sample Details table, select the pool icon to view details on the pool composition.
   Populates the RSB Volume (UL) and LIMS ID (Container) columns of the Sample Details table.
9. Select Next Steps. This selection triggers the Set Next Step automation. This automation sets the next step for samples to ADVANCE, advancing them to the next step in the protocol. The next step in the protocol is Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.0).
10. Select Finish Step.

At the end of this step, the pool of samples automatically advances to Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.0) step.

Step 2: Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.0)

In this step, scan the reagent cartridge barcode into Clarity LIMS, then manually place the working pool into the reagent cartridge for the NextSeq 1000/2000 run. This step validates the run setup and analysis information and generates sample sheet file and/or creates a planned run on IAP, depending on the Run Mode selected.

Automations

The following automations are configured on the step:

- Set Next Step
- Validate Reagent Cartridge Barcode
- Validate Run Setup and Create Planned Run
- Validate Single Input
- Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.0) automation configuration

These automations are described in the order in which they are triggered at run time.

Validate Single Input

Automatically triggered at the beginning of the step, this automation checks that there is only one container input to the step.

```
script:validateSampleCount -min 1 -max 1
```

Validate Reagent Cartridge Barcode

Automatically triggered on exit of the Placement screen, this automation does the following action:

- Validates the reagent cartridge barcode to make sure it conforms to the barcode mask [A-Za-z]{2}[0-9]{7}-[A-Za-z0-9]{4}:

```
if (!output.container.name.matches( ::[A-Za-z]{2}[0-9]{7}-[A-Za-z0-9]{4}:: ) )
{fail ( ::Invalid Reagent Cartridge Barcode. Please verify and try again.:: ) }
```

Validate Run Setup and Create Planned Run

Automatically triggered when a button on the Record Details screen is selected, this automation does the following actions:

- Validates the parameters entered on the Record Details screen, which are used to set up the run and generate the sample sheet file and/or create the planned run on IAP.
  - Run Name can only contain alphanumeric, dash, underscore, or period characters. Spaces are not permitted.

```
if (!step.::Run Name::.matches(::[a-zA-Z0-9-_.]+::))
```


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```
{ fail(::Run Name contains prohibited characters. Allowed characters are: a-z,
A-Z, 0-9, -, _, and .::) }
```

- Checks the Index Reads, Index Read 1, and Index Read 2 field values.
  - If Index Reads is No Index, Index Read 1 and Index Read 2 values must be zero (error results if it is otherwise).
  - If Index Reads is Single Index, Index Read 1 value must be greater than zero and Index Read 2 values must be zero (error results if it is otherwise).
  - If Index Reads is Dual Index, Index Read 1 and Index Read 2 values must be greater than zero (error results if it is otherwise).

```
if (step.::Index Reads:: == ::No Index::)
{ if (step.::Index Read 1:: != 0 || step.::Index Read 2:: != 0) { fail(::Index
Read 1 and Index Read 2 must be 0 if the Index Reads is No Index.::) } }
else
{ if (step.::Index Reads:: == ::Single Index::)
{ if (step.::Index Read 1:: == 0 || step.::Index Read 2:: != 0) { fail(::Index
Read 1 must be greater than 0 and Index Read 2 must be 0 if the Index Reads is
Single Index.::) } }
else
{ if (step.::Index Read 1:: == 0 || step.::Index Read 2:: == 0) { fail(::Index
Read 1 and Index Read 2 must be greater than 0 if the Index Reads is Dual
Index.::) } } }
```

- Checks the Paired End, Read 2 Cycles, and Index Read 2 field values.
  - If Paired End is set to True and Read 2 Cycles value is zero, an error is generated.
  - If Paired End is set to False and Read 2 Cycles or Index Read 2 values are greater than zero, an error is generated.

```
if (step.::Paired End::.toBoolean())
{ if (step.::Read 2 Cycles:: == 0) { fail(::Read 2 Cycles must not be zero if it
is Paired End read.::) }}
else
{if (step.::Read 2 Cycles:: != 0 || step.::Index Read 2:: != 0) {fail(::Read 2
Cycles and Index 2 Cycles must be 0 if it is not Paired End Read.:: )}}
```

- Checks the Adapter Sequence Read 1 and Adapter Sequence Read 2 field values.
  - Adapter Sequence Read 1 and Adapter Sequence Read 2 can only contain ACTG characters.

```
if (step.hasValue(::Adapter Sequence Read 1::) && !step.::Adapter Sequence Read
1::.matches(::[ACTG]+::)) { fail(::Adapter Sequence Read 1 contains prohibited
characters. Allowed characters are: ACTG::) };
if (step.hasValue(::Adapter Sequence Read 2::) && !step.::Adapter Sequence Read
2::.matches(::[ACTG]+::)) { fail(::Adapter Sequence Read 2 contains prohibited
characters. Allowed characters are: ACTG::) }
```


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- Check Override Cycles field value.
  - Override Cycles can only contain Y, N, I, U, 0-9, and semicolon characters.

```
if (step.hasValue(::Override Cycles::) && !step.::Override Cycles::.matches(::
[YNIU0-9;]+::))
```

```
{ fail(::Override Cycles contains prohibited characters. Allowed characters are:
Y, N, I, U, 0-9 and ;. Example: N1Y150;I8;I7N1;Y141U10.::) }
```

- Generates the sample sheet and/or create the planned run on IAP.
  - Sample sheet is attached to the step.

```
/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/SIS/v1/SISServices/extensions/automation/nextseq1k2
k-automation.jar
```

```
script:generate_v2_sample_sheet -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid2} -c {compoundOutputFileLuid0}
```

Set Next Step

Sets the next step for samples to ADVANCE, advancing them to the next step in the protocol—AUTOMATED - Run (NextSeq 1000/2000 Sequencing v1.0).

```
nextStep = ::ADVANCE::
```

Master Step Fields

There are 20 fields defined on the Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.0) step. These fields are required for sample sheet generation and planned run creation on IAP.

The fields are shown in the Configuration area (Custom Fields > Master Step Fields tab). See Table 5 for field configuration details.

Table 366 Table 5: Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.0) Master Step Fields Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Run Name	Text	• Required: Yes • Read Only: No	Not applicable
Instrument Type	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• NextSeq1000 • NextSeq2000

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Run Mode	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• Local • Hybrid • Cloud
Paired End	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• True • False
Read 1 Cycles	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 151 • 101 • 51
Read 2 Cycles	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• 151 • 101 • 51
Index Reads	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• No Index • Single Index • Dual Index
Index Read 1	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 0 • 6 • 8
Index Read 2	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 0 • 6 • 8
Analysis Workflow	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• GenerateFASTQ • None
Adapter Sequence Read 1	Text	• Required: No • Read Only: No	Not applicable
Adapter Sequence Read 2	Text	• Required: No • Read Only: No	Not applicable

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Barcode Mismatches Index 1	Numeric Dropdown	• Required: No • Read Only: No • Custom Entries: No	• 0 • 1 • 2
Barcode Mismatches Index 2	Numeric Dropdown	• Required: No • Read Only: No • Custom Entries: No	• 0 • 1 • 2
Override Cycles	Text	• Required: No • Read Only: No	Not applicable
Cloud Run ID	Text	• Required: No • Read Only: Yes	• Hidden - Used in creating planned run in IAP
Use Custom Index Read 1 Primer	Toggle Switch	• Required: No • Read Only: No	• Hidden Custom primer option is not supported at the moment. Do not enable as it causes integration to break.
Use Custom Index Read 2 Primer	Toggle Switch	• Required: No • Read Only: No	• Hidden Custom primer option is not supported at the moment. Do not enable as it causes integration to break.

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Use Custom Read 1 Primer	Toggle Switch	• Required: No • Read Only: No	• Hidden Custom primer option is not supported at the moment. Do not enable as it causes integration to break.
Use Custom Read 2 Primer	Toggle Switch	• Required: No • Read Only: No	• Hidden Custom primer option is not supported at the moment. Do not enable as it causes integration to break.

User Interaction

1. On the Queue screen, add the sample to the Ice Bucket and select View Ice Bucket.

   Only one sample is allowed.

   On entry to Placement screen, a validation checks if there is only one single input.
2. On the Ice Bucket screen, select Begin Work.
3. On the Placement screen:
   a. Drag the pool into the reagent cartridge in the Placed Samples area.
   b. Enter the barcode for the reagent cartridge by scanning or typing manually in the reagent container field.
   c. Select Record Details.

   On exiting the Placement screen, the Validate Reagent Cartridge Barcode automation checks that the reagent cartridge barcode conforms to the barcode mask [A-Za-z]{2}[0-9]{7}-[A-Za-z0-9]{4}. If not, an error message displays and you are blocked from proceeding.

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4. On the Record Details screen, the Reagent Lot Tracking section tracks the NextSeq 1000/2000 Reagent Cartridge used in the step. Select from the active lots displayed in each drop-down list.

   > To make reagent lots available for selection in the Reagent Lot Tracking section, configure and activate them. Do this step on the Configuration > Consumables screen.

   The fields displayed on the Run and Analysis Configuration section are used to set up the run and generate the sample sheet file.
   Some of these fields are autopopulated and some must be completed manually:

   - Run Name—Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.
   - Instrument Type—Select from preset options: NextSeq 1000 or NextSeq 2000.
   - Run Mode—Select from preset options: Local, Hybrid, Cloud. See the *Run Mode Details* section for more information on different run mode.
   - Paired End—Select from preset options: True or False.
   - Read 1 Cycles—Select from preset options (151, 101, 51) or type a custom value.
   - Read 2 Cycles—Select from preset options (151, 101, 51) or type a custom value.
   - Index Reads—Select from preset options: No Index, Single Index, Dual Index.
   - Index Read 1—Select from preset options (0, 6, 8) or type a custom value.
   - Index Read 2—Select from preset options (0, 6, 8) or type a custom value.
   - Analysis Workflow—Select from preset options: GenerateFASTQ or None. See the *Analysis Workflow Details* section for more information.
   - Adapter Sequence Read 1—Enter the Read 1 adapter sequence of the index adapter kit. Not required if Analysis Workflow is None.
   - Adapter Sequence Read 2—Enter the Read 2 adapter sequence of the index adapter kit. Not required if Analysis Workflow is None.
   - Barcode Mismatches Index 1—[Optional] Select from preset options: 0, 1, 2. Leave it blank if you are unsure.
   - Barcode Mismatches Index 2—[Optional] Select from preset options: 0, 1, 2. Leave it blank if you are unsure.
   - Override Cycles—[Optional] String used to specify UMI cycles and mask out cycles of a read (eg, N1Y150;I8;I7N1;Y141U10). Leave it blank if you are unsure.

5. On the Record Details screen, select Validate Run Setup and Create Planned Run.
   The automation script performs the following actions:

   - Validates the parameters entered on the Record Details screen.
   - Creates the planned run and sends it to Illumina Analytics Platform (IAP) if Run Mode is Hybrid or Cloud.

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- Generates the sample sheet and attaches it to the placeholder in the Files area of the Record Details screen for all Run Mode types.

  For Local run mode, download a copy of the sample sheet. It is used to start the sequencing run on the instrument.

6. Select Next Steps.
   On the Assign Next Steps screen, the next step for samples is set to the next step in the workflow—AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.0).
7. Select Finish Step.

At the end of this step, the pool of samples automatically advances to and is queued for the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.0) step.

Run Mode Details

The run mode applies to all runs and determines how the run and analysis parameters are configured and how to analyze data.

- Local Mode—NextSeq 1000/2000 Integration service generates the sample sheet. Run/Analysis configuration is imported manually into NextSeq 1000/2000 instrument via a sample sheet to start the run. The downstream secondary analysis is done using the onboard DRAGEN module.
- Hybrid Mode—NextSeq 1000/2000 Integration service sends the run/analysis configuration to Illumina Analytics Platform (IAP). Run/Analysis configuration is downloaded to NextSeq 1000/2000 instrument via IAP to start the run. The downstream secondary analysis is done using the onboard DRAGEN module.
- Cloud Mode—NextSeq 1000/2000 Integration service sends the run/analysis configuration to Illumina Analytics Platform (IAP). Run/Analysis configuration is downloaded to NextSeq 1000/2000 instrument via IAP to start the run. The downstream secondary analysis is performed in the cloud.

Analysis Workflow Details

The analysis workflow selection determines if the sample is advanced to the Demultiplexing (NextSeq 1000/2000 Sequencing v1.0) step or completed at the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.0) step.

- GenerateFASTQ—After the sequencing run completes, NextSeq 1000/2000 integration service automatically completes AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.0) step and advances the samples to the Demultiplexing (NextSeq 1000/2000 Sequencing v1.0) step.
- None—None is selected to skip the Demultiplexing (NextSeq 1000/2000 Sequencing v1.0) step. NextSeq 1000/2000 integration service automatically completes AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.0) and removes the samples from the workflow.

Step 3: AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.0)

Do not add samples to the Ice Bucket or start or complete the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.0) step. The integration does this step automatically.

This step is fully automated.

The integrations start the step automatically and data from the run is parsed back to Clarity LIMS. No user interaction is required. In this step, the pooled samples in the reagent cartridge are sequenced in the NextSeq 1000/2000 instrument.

The following automations are configured on the step:

- Set Next Step
  - The Set Next Step automation is the only automation configured on this step. It automatically sets the samples to advance to the next step—Demultiplexing (NextSeq 1000/2000 Sequencing v1.0).

```
nextStep = ::ADVANCE::
```

Master Step Fields

There are 16 fields defined on the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.0) step. These fields are used to display the run status and sequencing run and analysis configuration parsed from the `RunParameters.xml` file of the sequencing run.

Do not modify the name of any of the master step fields. It causes the integration to break.

See Table 6 for how some of these step fields map to the fields on the `RunParameters.xml` file.

Table 367 Table 6: Run Parameters and Corresponding Clarity LIMS Step Fields

Master Step Field	RunParameters.xml Field
Current Cycle	Calculated based on CompletedCycles Field
Current Read	Calculated based on CompletedCycles field against PlannedCycles
Flow Cell ID	FlowCellSerialNumber
Flow Cell Lot Number	FlowCellLotNumber
Instrument Control Software Version	ApplicationVersion
Instrument ID	InstrumentSerialNumber
Output Folder	OutputFolder
Reagent Cartridge ID	CartridgeSerialNumber
Reagent Cartridge Lot Number	CartridgeLotNumber

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Master Step Field	RunParameters.xml Field
RTA Version	RtaVersion
Run Name	ExperimentName
Secondary Analysis Workflow	SecondaryAnalysisWorkflow

Global Fields

The following global fields are used to capture the run metrics in Clarity LIMS.

- % Bases >=Q30 R1
- % Bases >=Q30 R2
- % Error Rate R1
- % Error Rate R2
- Yield (Gb) R1
- Yield (Gb) R2
- Reads PF R1
- Reads PF R2
- %PF R1
- %PF R2
- % Aligned R1
- % Aligned R2
- % Phasing R1
- % Phasing R2
- % PrePhasing R1
- % PrePhasing R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- Cluster Density R1
- Cluster Density R2

At the end of this step, the pool of samples undergoes either one of the two following actions:

1. If the Analysis Workflow UDF in Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.0) step is set to GenerateFASTQ, the pool automatically advances to and is queued for the Demultiplexing (NextSeq 1000/2000 Sequencing v1.0) step.

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2. If the Analysis Workflow UDF in Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.0) step is set to None, the pool automatically completes the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.0) step and gets removed from the workflow.

Step 4: Demultiplexing (NextSeq 1000/2000 Sequencing v1.0)

Do not add samples to the Ice Bucket or start the Demultiplexing (NextSeq 1000/2000 Sequencing v1.0) step. The integration does this action automatically.

This step is a semiautomated step.

The integration starts the step automatically and demultiplexing data from the GenerateFASTQ secondary analysis is parsed back to Clarity LIMS. Then review the demultiplexing result parsed into Clarity LIMS, assign QC flags, and complete the step.

Automations

The following automations are configured on the step:

- Assign Demultiplexing QC flags—Automatically assigns QC flags based on the criteria set on the step fields.
- Set Next Step—Automatically sets the next step to Advance and the samples complete the protocol.

Master Step Fields

There are eight fields defined on the Demultiplexing (NextSeq 1000/2000 Sequencing v1.0) step.

Table 7 lists the master step fields that are configured on the Demultiplexing (NextSeq 1000/2000 Sequencing v1.0) step.

Table 368 Table 7: Master Step Field Configuration for Demultiplexing (NextSeq 1000/2000 Sequencing v1.0) Step

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Criteria 1 - Source Data Field	Text Dropdown	• Required: No • Read Only: No • Custom Entries: Yes	• # Reads • # Perfect Index Reads • # One Mismatch Index Reads • # of >= Q30 Bases (PF)

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Criteria 2 - Source Data Field	Text Dropdown	• Required: No • Read Only: No • Custom Entries: Yes	• # Reads • # Perfect Index Reads • # One Mismatch Index Reads • # of >= Q30 Bases (PF)
Criteria 1 - Operator	Text Dropdown	• Required: No • Read Only: No • Custom Entries: Yes	• >= • <= • = • !=
Criteria 2 - Operator	Text Dropdown	• Required: No • Read Only: No • Custom Entries: Yes	• >= • <= • = • !=
Criteria 1 - Threshold Value	Numeric	• Required: No • Read Only: No	• Valid integer value
Criteria 2 - Threshold Value	Numeric	• Required: No • Read Only: No	• Valid integer value
Log	Multiline Text	• Required: No • Read Only: Yes	Not applicable
Reagent Cartridge ID	Text	• Required: No • Read Only: Yes	• Hidden • Used for parsing demultiplexing results

Global Fields

There are four fields defined on the Demultiplexing (NextSeq 1000/2000 Sequencing v1.0) step. which are used to display the demultiplexing result metrics for individual library in the sample pool.

Table 8 lists the global fields that are configured on the Demultiplexing (NextSeq 1000/2000 Sequencing v1.0) step.

Table 369 Table 8: Global Field Configuration for Demultiplexing (NextSeq 1000/2000 Sequencing v1.0) Step

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
# of >= Q30 Bases (PF)	Numeric	• Required: No • Read Only: Yes	Not applicable
# One Mismatch Index Reads	Numeric	• Required: No • Read Only: Yes	Not applicable
# Perfect Index Reads	Numeric	• Required: No • Read Only: Yes	Not applicable
# Reads	Numeric	• Required: No • Read Only: Yes	Not applicable

User Interaction

1. On the Record Details screen, complete the following fields to define the QC Passed or QC Failed criteria for the demultiplexing result:
   - Criteria 1 - Source Data Field—Select the first source data field criteria type. Valid options include: # Reads, # Perfect Index Reads, # One Mismatch Index Reads, # of >= Q30 Bases (PF).
   - Criteria 2 - Source Data Field—Select the second source data field criteria type. Valid options include: # Reads, # Perfect Index Reads, # One Mismatch Index Reads, # of >= Q30 Bases (PF).
   - Criteria 1 - Operator—Select the operator that defines the range of values to be included in the first criteria source data field. Valid options include: >=, <=, =, !=.
   - Criteria 2 - Operator—Select the operator that defines the range of values to be included in the second criteria source data field. Valid options include: >=, <=, =, !=.
   - Criteria 1 - Threshold Value—Select a threshold value for the source data field 1.
   - Criteria 2 - Threshold Value—Select a threshold value for source data field 1.
2. On the Record Details screen, select Assign Demultiplex QC flags. The automation script does the following action:
   - Assigns QC flags to each individual library based on the criteria set in the step fields.

     It is optional to use Assign Demultiplex QC flags. You can manually assign QC flag through the QC column in Sample Details table.
3. Select Next Steps.
   On the Assign Next Steps screen, the Next Step field for all samples is prepopulated with Mark protocol as complete.

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4. Select Finish Step to complete the workflow.

At the end of this step, the whole protocol is completed. Samples are removed from the workflow.

How the NextSeq 1000/2000 Integration Works

Sample Sheet Generation and Planned Run Creation

On the Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.0) step, when the Validate Run Setup and Create Planned Run automation is triggered, all the run and analysis parameters that were entered in the Run Details screen are sent to Illumina Analytics Platform (IAP). IAP validates the run and analysis configuration and sends an error message to Clarity LIMS if the validation failed. If the validation passes, IAP generates the sample sheet (and sends back to Clarity LIMS) and/or creates a planned run depending on the Run Mode selected.

For Local run mode, only the sample sheet is generated and stored on Clarity LIMS. The sample sheet contains all the run and analysis configuration required to start the run on the instrument.

For Hybrid/Cloud run mode, a planned run is created on IAP. This planned run contains all the run and analysis configuration required to start the run on the instrument. The sample sheet is also generated and stored on Clarity LIMS for reference purpose.

Run Status, Primary Metrics and Analysis Results Parsing and Recording

After the sequencing run is started on the instrument, NextSeq 1000/2000 Control Software notifies BaseSpace Sequence Hub (BSSH) that the sequencing run has been started.

BSSH then does the following actions:

- For Local run mode, create a new run on the BSSH database with the run configuration information received from NextSeq 1000/2000 Control Software.
- For Hybrid/Cloud run mode, create a new run on the BSSH database by copying the run configuration information from the planned run created in IAP, using a special Cloud Run ID received from NextSeq 1000/2000 Control Software.

After the run has been created on BSSH, a run update event message that carries the RunStarted equivalent message is created on BSSH and delivered through IAP and, eventually, to Clarity LIMS SQS queue service. The message is grabbed and processed by SIS integration service.

Based on the reagent cartridge barcode information in the event message (see the following example), SIS looks in the queue of AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.0) step for a container with the same reagent cartridge barcode. When identified, the step is started automatically and the Run Status UDF on Record Details screen is updated to RunStarted. SIS then updates all the other step UDFs (eg, Current Read, Output Folder etc.) by parsing the details from the RunParameters.xml uploaded to BSSH by NextSeq 1000/2000 Control Software.

```
#Example of an run update event
{
    "acl": [
```


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```
        "wid:61dc14e8-d823-3bd1-bea3-66f9c8388254"
    ],
    "apiUrl":
    "https://api.basespace.illumina.com/v2/runs/r.ZvTfDXsNhUyh2kA5S4Zewg",
    "dateModified": "2020-03-26T09:19:58.5546497Z",
    "flowcellBarcode": "11M11COMC",
    "gdsFolderPath": "/Runs/200326_MP2-15_87_11M11COMC_r.ZvTfDXsNhUyh2kA5S4Zewg",
    "gdsVolumeName": "bssh.61dc14e8d8233bd1bea366f9c8388254",
    "id": "r.ZvTfDXsNhUyh2kA5S4Zewg",
    "instrumentRunId": "200326_MP2-15_87_11M11COMC",
    "name": "200326_MP2-15_87_11M11COMC",
    "reagentBarcode": "EC0001990-EC5",
    "sampleSheetName": "SampleSheet.csv",
    "status": "Running",
    "v1pre3Id": "194424236"
}
```


NextSeq 1000/2000 Control Software continues to notify BSSH of any run status change throughout the course of the sequencing run, and the same run update event delivery process follows.

Table 9 lists out all the run status and how they are being handled by SIS integration service.

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Table 370 Table 9: Run Status and How SIS Integration Service Handles the Status

Run Status	Shown in Step	Details

RunStarted	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.0)	• Run Status UDF is updated to RunStarted.
		• All other step UDFs are updated based on RunParameters.xml detail obtained from BSSH.
		• Reagent cartridge lot information received from BSSH is compared against the details of the preaccessioned reagent cartridge on Clarity LIMS.
		  • If the expiration date is different, it is updated to the date received from BSSH.
		  • If reagent cartridge is not found, it is created and set to Active.
		  • If reagent cartridge is not in Active status, it is set to Active.
		• Multiline Sequencing Log UDF is updated.

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Run Status	Shown in Step	Details
RunCompletedSuccessfully	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.0)	• Run Status UDF is updated to RunCompletedSuccessfully • Current Read and Current Cycle UDFs is updated to the final cycle number and read number based on `RunParameters.xml` detail obtained from BSSH. • Sequencing run result (primary metrics) is downloaded from BSSH and the fields in Sample Details table (eg, % Error Rate R1, % Phasing, etc.) are updated with the values. • Multiline Sequencing Log UDF is updated. • SIS autocompletes the step and routes the samples in the reagent cartridge container to the next step (ie, Demultiplexing (NextSeq 1000/2000 Sequencing v1.0) step if GenerateFastQ is selected as the Analysis Workflow in Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.0) step). Otherwise, the step is completed automatically and the samples are removed from the workflow.

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Run Status	Shown in Step	Details
RunErroredOut	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.0)	• Run Status UDF is updated to RunErroredOut • Current Read and Current Cycle UDFs are updated to the final cycle number and read number that the run stopped based on RunParameters.xml detail obtained from BSSH. • Sequencing run result (primary metrics) is downloaded from BSSH and the fields in Sample Details table (eg, % Error Rate R1, % Phasing, etc.) are updated with the values. • Multiline Sequencing Log UDF is updated. • SIS does not autocomplete the step. Complete the step manually.
RunAbortedByUser	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.0)	• Run Status UDF is updated to RunAbortedByUser • Current Read and Current Cycle UDFs are updated to the final cycle number and read number that was aborted based on RunParameters.xml details obtained from BSSH. • Sequencing run result (primary metrics) is downloaded from BSSH and the fields in Sample Details table (eg, % Error Rate R1, % Phasing, etc.) are updated with the values. • Multiline Sequencing Log UDF is updated. • SIS does not autocomplete the step. Complete the step manually.
AnalysisStarted	Demultiplexing (NextSeq 1000/2000 Sequencing v1.0)	• SIS starts the step automatically for the reagent cartridge container. • Multiline Sequencing Log UDF is updated.

Run Status	Shown in Step	Details
AnalysisCompleted	Demultiplexing (NextSeq 1000/2000 Sequencing v1.0)	• SIS downloads the Demultiplex_ stats.csv file, which contains the demultiplexing result from BSSH. • The file is parsed and details are recorded in the Sample Details table for each library in the library pool. • Multiline Sequencing Log UDF is updated. • SIS does not autocomplete the step. Assign the QC flag to the individual library and manually complete the step.

Step-by-Step Instruction to Start A Sequencing Run On Instrument With Different Run Modes

Local Run Mode

1. On the NextSeq 1000/2000 Control Software interface, select the menu button and select Settings.
2. Select Local Run Setup and Proactive, Run Monitoring and Storage*.
3. [Optional] Enter the default output folder location.
4. Select Save.
5. On the main screen, select Start.
6. Sign in to the BaseSpace Sequence Hub (BSSH) account that was used during NextSeq 1000/2000 Integration package installation setup.
   a. Select the workgroup (if applicable).
   b. Select Next.
7. Select Choose, and a file browser pops up.
8. Browse and select the sample sheet generated during the Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.0) step.
   When selected, the sample sheet has its content validated. After the validation passes, run and analysis configuration is parsed and autopopulated in the next page.
9. Select Review to review the sequencing run settings.
10. Select Load and manually load the reagent cartridge and flow cell.
11. Select Sequence and the pre-run check proceeds.
    After the pre-run check passes, the sequencing run starts automatically.

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Hybrid or Cloud Run Mode

1. On the NextSeq 1000/2000 Control Software interface, select the menu button and select Settings.
2. Select Online Run Setup and Proactive, Run Monitoring and Storage*.
3. [Optional] Enter the default output folder location.
4. Select Save.
5. On the main screen, select Start.
6. Sign into the BaseSpace Sequence Hub (BSSH) account that was used during NextSeq 1000/2000 Integration package installation setup.
   a. Select the workgroup (if applicable).
   b. Select Next.
   Planned runs that were created in the Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.0) step, using Hybrid or Cloud run mode, show up here.
7. Select the run and select Review to review the sequencing run settings.
8. Select Load and manually load the reagent cartridge and flow cell.
9. Select Sequence and the pre-run check proceeds.
   After the pre-run check passes, the sequencing run starts automatically.

Components Installed with this Integration

The following sections describe the various components that are installed by default as part of this integration. These components include files, properties, reagent categories/label groups, reagent kits, control types, and containers. Information on installed workflows, protocols, steps, and automation points is provided in the *Workflows, Protocols, and Steps* section.

The Illumina NextSeq 1000/2000 Integration v1.0 is distributed as two RPM packages:

- BaseSpaceLIMS-SIS-Core
- BaseSpaceLIMS-SIS-Nextseq1k2k

BaseSpaceLIMS-SIS-Core is a shared component with all other (future) integrations using IAP. BaseSpaceLIMS-SIS-Nextseq1k2k, as an extension, contains NextSeq 1000/2000 specific integration logic. This logic can be skipped if customers do not have NextSeq 1000/2000 instruments. These two RPM packages must be installed on the Clarity LIMS server.

The BaseSpaceLIMS-SIS-Core RPM installs the following:

- SIS Core JAR file
- SIS Core Automation JAR file
- Configuration file—`application.properties`
- Three configuration scripts:
  - `configure_sis_core-v1.sh`

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  - `configure_sequence_hub_access_token-v1.sh`
  - `configure_sis_remove_subscription.sh`
- Two execution scripts
  - `sis_core`
  - `run_sis_core`

The base installation folder is /opt/gls/clarity/extensions/SIS/v1

The BaseSpaceLIMS-SIS-Nextseq1k2k RPM installs the following:

- SIS Extension JAR file for NextSeq 1000/2000
- NextSeq 1000/2000 Automation JAR file
- NextSeq 1000/2000 Configuration file—`nextseq1k2k.properties`
- Configuration script—`configure_nextseq1k2k-v1.sh`

The base installation folder is /opt/gls/clarity/extensions/SIS/v1/SISServices/extensions

The NextSeq 1000/2000 integration protocols configuration is delivered through the Illumina Preset Protocols (IPP) v1.0, which requires installation of the NGS Extensions Package (v5.19 or later).

- If the NGS Extensions Package is not already installed, or if a version earlier than v5.19 is installed, the latest version is installed by default with the NextSeq 1000/2000 integration. See Release Notes for details.
- If NGS Extensions Package v5.19 or later is already installed, upgrade is not forced or required.

SIS Core Configuration File

The `application.properties` configuration file is installed to:

```
/opt/gls/clarity/extensions/SIS/v1/SISServices/conf/application.properties
```

Do not change any of the fields in the configuration file on your own, as it risks breaking the integration.

It is configured by script and the list of properties include:

- Script configurable properties

Property	Description	Default Value
sequenceHubApiUri	BaseSpace Sequence Hub API URL	https://api.basespace.illumina.com
iapBaseApiUri	IAP API URL	https://use1.platform.illumina.com
iapApiKey	IAP API KEY that is used to enable user to access to IAP	Not applicable

Property	Description	Default Value
workgroupId	Workgroup ID of the BaseSpace Sequence Hub Login Account (Public user does not need this property)	Not applicable

- Script-generated properties

Property	Description
iapAccessToken	IAP Access Key generated
sequenceHubAccessToken	BaseSpace Sequence Hub Access Token to access BaseSpace API
subscriptionId	Subscription ID of subscription that created to subscribe Run Status Change from IAP

- System properties

Property	Description	Default Value
sqsPollingInterval	The interval of the main SIS scheduler job to execute. It is in the unit of seconds.	60
maxNumberOfMessages	The maximum number of messages pulled from SQS in one pull.	10
receiveMessageWaitTimeSeconds	The maximum waiting time in seconds when pulling from SQS.	20
maxMessageHandlingRetryTimes	The maximum of message handling retry times in case of failure.	3
runEvents	The key in the stored failure message JSON file.	runEvents

Extension Configuration File

The `nextseq1k2k.properties` configuration file is installed to:

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```
/opt/gls/clarity/extensions/SIS/v1/SISServices/extensions/conf/nextseq1k2k.properties
```

Do not change any of the fields in the configuration file. It risks breaking the integration.

It is configured by script and the list of properties included:

- Script configurable properties

Property	Description	Default Value
automatedStepNames	The sequencing run step name that is supported by NextSeq1k2k extension. Comma separated.	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.0)
analysisStepNames	The demultiplexing step name that is supported by NextSeq1k2k extension. Comma separated.	Demultiplexing (NextSeq 1000/2000 Sequencing v1.0)
containerType	The supported container type used in NextSeq1k2k workflow step Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.0).	NextSeq 1000/2000 Reagent Cartridge

- Script-generated properties

Property	Description	Default Value
reagentBarcodeRegex	The reagent barcode regex that is supported by NextSeq1k2k extension.	[A-Za-z]{2}[0-9]{7}-[A-Za-z0-9]{4}
cloudFastqAvdId	In Cloud run mode, the planned run created and sample sheet generated depends on this value.	avd.042b19e86d5646bdb0520c4faa13f411

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Property	Description	Default Value
localFastqAvdId	In Hybrid run mode, the planned run created and sample sheet generated depends on this value.	avd.d7e7858a351948708fd6c15e7298d7db
analysisAppApplicationFamilySlug	In Cloud run mode, it is used to retrieve the demultiplexing stats data from BaseSpace Sequence Hub	illumina-inc.basespace-dragen-analysis
maxWaitingTimeInSeconds	The maximum of waiting time in seconds to retrieve RunParameter.xml from BaseSpace Sequence Hub.	180*60 (180 mins)

Reagent Categories/Label Groups

- TruSeq HT Adapters v2 (D7-D5)

Reagent Kits

- Resuspension Buffer (RSB)
- NextSeq 1000/2000 Reagent Cartridge

Container Types

- Tube
- NextSeq 1000/2000 Reagent Cartridge

Control Types

- PhiX v3

This integration supports:

- NextSeq 1000/2000 Reagent Cartridge with barcode provided in the format [A-Za-z]{2}[0-9]{7}-[A-Za-z0-9]{4}
  - Example: EC1234567-EC03

Rules and Constraints

- The workflow configuration contains several validation checks. To make sure that the calculations work properly, it is important that you do not disable any of this validation logic. The validation checks determine:
  - Which samples, and how many, can enter each step together.
  - Which samples, and how many, can be pooled together.
- The NextSeq 1000/2000 reagent cartridge barcode must be unique. There should not be multiple NextSeq 1000/2000 reagent cartridge containers in the system with the same name.
- Reagent labels (indexes) must be unique.
- One library pool can only contain one library or control with no label (index).
- Do not manually start or complete the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.0) step. This step is a fully automated step, and the SIS integration service does not update samples correctly if they have been manually started.
- Do not manually start the Demultiplexing (NextSeq 1000/2000 Sequencing v1.0) step. This step is semiautomated, and the SIS integration service does not update the demultiplexing result correctly if they have been manually started.
- For the automated run to start successfully, select Validate Run Setup and Create Planned Run.

NextSeq 1000/2000 Integration v1.0 Validation and Troubleshooting

This section explains how to validate the installation of the Illumina NextSeq 1000/2000 Integration Package v1.0.

The validation process involves:

- Running samples through the Library Prep Validation v2.0 workflow.
  - The workflow contains a single-step protocol that models the library prep workflow required to produce libraries tagged with index sequences. At the end of the step, these libraries are routed to NextSeq 1000/2000 Sequencing v1.0 workflow.
- Running the libraries through the NextSeq 1000/2000 Sequencing v1.0 workflow. This process validates the following information:
  - Successful sequential step advancement of samples in the following steps:

1. Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.0)
2. Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.0)
3. AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.0)
4. [Optional] Demultiplexing (NextSeq 1000/2000 Sequencing v1.0)

- Automatic validation of run setup information before sample sheet generation or planned run creation.
- Automated generation of a sample sheet. The sample sheet is used to start the sequencing run on NextSeq 1000/2000 Control Software via Local run mode.
- Automated creation of a planned run on Illumina Analytics Platform (IAP). The planned run created is retrieved by NextSeq 1000/2000 Control Software to start run via Hybrid or Cloud run mode.

- Automated tracking of the NextSeq 1000/2000 sequencing run and parsing of run statistics from BaseSpace Sequence Hub (BSSH) into Clarity LIMS using SIS Integration Service.

Before running the validation steps below, these steps assume that the NextSeq 1000/2000 Sequencing v1.0 Integration Package is installed and you have imported the default Clarity LIMS configuration.

Validation Steps

Activate Workflow, Create Project, Add and Assign Samples

The following steps set up Clarity LIMS in preparation for running samples through the Library Prep Validation v2.0 and NextSeq 1000/2000 Sequencing v1.0 workflows.

Step 1: Activate the workflow

In the Configuration tab of Clarity LIMS under Workflows, activate both the Library Prep Validation v2.0 and NextSeq 1000/2000 Sequencing v1.0 workflows.

Step 2: Create a Project, Add Samples

1. On the Projects and Samples screen, create a project and add samples to it.
2. Assign the samples to the Library Prep Validation v2.0 workflow.

Library Prep Protocol: Library Prep Validation v2.0

This single-step protocol models the library prep required to produce libraries tagged with index sequences that are ready for the NextSeq 1000/2000 Sequencing v1.0 workflow.

Run Library Prep Validation v2.0 Step

1. In Lab View, locate the Library Prep Validation v2.0 protocol. The samples are queued for the Library Prep Validation v2.0 step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.

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3. On the Ice Bucket screen, select the label groups to use.
   - TruSeq HT Adapters v2 (D7-D5) is the default label group delivered by Library Prep Validation v2.0 protocol.
   - For other reagent labels (indexes), manually create them in the Configuration screen under Consumables → Labels.

   For more information on how to create reagent label group, refer Clarity LIMS documentation.
4. Select Begin Work.
5. On the Placement screen:
   a. In the Placed Samples area in the Container field, scan or enter the barcode of the destination container.
   b. Select the samples from the Samples to be Placed area, then drag them over to the container wells on the right.
   c. Select Add Labels.
6. On the Add Labels screen, the Labels to Add list is populated with labels from the TruSeq HT Adapters v2 (D7-D5) label group. This label group is the only one configured in the out-of-the-box integration. It is used by default in this step.
   a. Select labels on the left, and drag them over to the container on the right to assign them to placed samples.
   b. Select Record Details.
7. On the Record Details screen:
   a. In the Sample Details table, enter values for Molarity (nM) for all the individual sample. Type values individually or use the copy/paste functionality to enter multiple values from a spreadsheet.
      - Molarity (nM) values are used in Calculate Volume automation script in Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.0) step.
   b. The Sequencing Instrument column indicates the instrument to be used for the run. Select NextSeq 1000/2000.
   c. Populate the fields in this column using one of the following methods:
      - To populate fields individually for each sample, select from the drop-down list in each row.
      - To populate fields all at one time, use the Copy and Paste buttons to enter multiple values from a spreadsheet (see the following note).

   Alternatively, select inside the table and press Ctrl + A to select all rows. At the top of the table, select Sequencing Instrument, then select NextSeq 1000/2000 from the adjacent drop-down list. Select Apply.

   If you are working with many samples, you can use the copy/paste functionality to enter multiple values from a spreadsheet.

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a. Select the Copy button to copy the sample information from Clarity LIMS to the clipboard.
b. Open a blank Excel document and paste the contents of the clipboard into the spreadsheet.
c. In the spreadsheet, enter the Molarity (nM) and Sequencing Instrument values. Select the entire spreadsheet, including the headers, (Ctrl + A) and copy (Ctrl + C) the information to the clipboard.
d. In Clarity LIMS, select the Paste button. Paste the contents of the clipboard (Ctrl + V) into the Update Table window and then select Update to paste into the Sample Details table.

8. Select Next Steps.
   This selection triggers the Next Steps automation, which sets the value of the next step (for all samples) to Remove from workflow. The Routing Script automation expects this value and requires it to advance samples to the next step successfully.
9. On the Assign Next Steps screen in the Samples table, the Next Step for all samples is prepopulated with Remove from workflow.

   Do not change this value. If Next Step is not set to Remove from workflow, the routing script is not able to route samples correctly.

10. Select Finish Step.
    On exit from the step, the Routing Script automation is triggered. This automation assigns samples to the first step of the NextSeq 1000/2000 Sequencing v1.0 workflow, which is Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.0) step.

Protocol: NextSeq 1000/2000 Sequencing v1.0

This protocol consists of four steps:

1. Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.0)
2. Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.0)
3. AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.0)
4. Demultiplexing (NextSeq 1000/2000 Sequencing v1.0)

Step 1: Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.0)

1. In Lab View, locate the NextSeq 1000/2000 Sequencing v1.0 protocol. The samples are queued for the Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.0) step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Pooling screen:
   a. Create a pool by dragging samples into the Pool Creator.

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b. Type a name for the pool or accept the default name (Pool #1).

c. Select Record Details.

5. On the Record Details screen, the Reagent Lot Tracking section tracks the Resuspension Buffer (RSB) lot information used in the step. You might need to add and activate lots for RSB.
   To add and activate reagent lots:
   a. In a new browser tab, open an additional instance of Clarity LIMS.
   b. Navigate to the Consumables configuration screen.
   c. Select the RSB reagent kit and select New Lot.
   d. Enter the lot details and select Save.
   e. Set the Status of Reagent Lot toggle switch to Active.
6. In the Reagent Lot Tracking section, select from the active lots displayed in the drop-down list.
7. The Step Details area contains two required fields:
   - Final Loading Concentration (pM)—The value entered in this field is the recommended final loading concentration from NextSeq 1000/2000 System Guide depending on the library type. There is a default drop-down list of 650, 750, 1000 and 2000. A custom value is acceptable.
   - Final Loading Volume (µl)—The value in this field is the final loading volume of the pool into the reagent cartridge. The field is prepopulated with the configured default value, 24 µl, which is recommended by Illumina NextSeq 1000/2000 Instrument System Guide. However, it can be edited if more volume is necessary.
8. Select Calculate Volumes. This selection triggers the Calculate Volumes automation. This automation calculates the volume required for each library to form a pool that has the concentration and volume specified in the step details fields.
   The automation also generates the Calculation File (CSV) and attaches it to the step. This file contains volume information of each of the samples and RSB buffer to add to the pool. Select the file to download it, then open it in Excel.
9. In the Sample Details table, select the pool icon to view details on the pool composition.
10. Select Next Steps. This selection triggers the Set Next Step automation, which sets the next step for samples to ADVANCE, advancing them to the next step in the protocol. The next step is Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.0).
    On the Assign Next Steps screen, the next step for samples is already set to the next step in the workflow. The next step is Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.0).
11. Select Finish Step.

At the end of this step, the pool of samples automatically advances to Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.0) step.

Step 2: Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.0)

1. In Lab View, locate the NextSeq 1000/2000 Sequencing v1.0 protocol. The pool of samples is queued for the Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.0) step.

2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
   The Validate Single Input automation is triggered. This automation checks that there is only one container input to the step.
4. On the Placement screen:
   a. Drag the pool into the NextSeq 1000/2000 Reagent Cartridge in the Placed Samples area.
   b. Scan or type the barcode of the reagent cartridge into the NextSeq 1000/2000 Reagent Cartridge field.
   c. Select Record Details.
   On exit of the Placement screen, the Validate Reagent Cartridge Barcode automation checks that the reagent cartridge barcode conforms to the barcode mask [A-Za-z]{2}[0-9]{7}-[A-Za-z0-9]{4}. If not, an error message displays.
5. On the Record Details screen, the Reagent LotTracking section tracks the NextSeq 1000/2000 Reagent Cartridge lot information used in the step. Follow the steps in Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v1.0) step if you need to add a new lot.
6. In the Reagent Lot Tracking section, select from the active lots displayed in the drop-down list.
7. The fields displayed on the Record Details screen are used to create planned run and generate the sample sheet file.
   - Run Name—Enter the experiment name. Only alphanumeric characters, dashes, and underscores are permitted. Spaces are not permitted.
   - Instrument Type—Select from preset options: NextSeq 1000, NextSeq 2000.
   - Run Mode—Select from preset options: Local, Hybrid, Cloud. See *Run Mode Details* for more information on different run mode.
   - Paired End—Select from preset options: True or False.
   - Read 1 Cycles—Select from preset options (151, 101, 51) or type a custom value.
   - Read 2 Cycles—Select from preset options (151, 101, 51) or type a custom value.
   - Index Reads—Select from preset options: No Index, Single Index, Dual Index.
   - Index Read 1—Select from preset options (0, 6, 8) or type a custom value.
   - Index Read 2—Select from preset options (0, 6, 8) or type a custom value.
   - Analysis Workflow—Select from preset options: GenerateFASTQ or None. See *Analysis Workflow Details* for more information.
   - Adapter Sequence Read 1—[Optional] Enter the Read 1 adapter sequence of the index adapter kit.
   - Adapter Sequence Read 2—[Optional] Enter the Read 2 adapter sequence of the index adapter kit.

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- Barcode Mismatches Index 1—[Optional] Select from preset options: 0, 1, 2. Leave it blank if you are unsure.
- Barcode Mismatches Index 2—[Optional] Select from preset options: 0, 1, 2. Leave it blank if you are unsure.
- Override Cycles—[Optional] String used to specify UMI cycles and mask out cycles of a read, eg, N1Y150;I8;I7N1;Y141U10. Leave it blank if you are unsure.

8. On the Record Details screen, select Validate Run Setup and Create Planned Run. This selection triggers the automation script, which does the following actions:
   - Validates the parameters entered on the Record Details screen.
   - Create the planned run and send it to Illumina Analytics Platform (IAP) if Run Mode is Hybrid or Cloud.
   - Generates the sample sheet and attaches it to the placeholder in the Files area of the Record Details screen for all Run Mode types.
9. Select Next Steps.
   On the Assign Next Steps screen, the next step for samples is set to the next step in the workflow. The next step is AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.0).
10. Select Finish Step.

At the end of this step, the pool of samples automatically advances to and is queued for the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.0) step. The sequencing run is ready to be started. For details on how to start the sequencing run for different run modes, refer to *NextSeq 1000/2000 Integration v1.0 User and Configuration Guide*.

Run Mode Details

The run mode applies to all runs and determines how the run and analysis parameters are configured and how to analyze data.

Local Mode

NextSeq 1000/2000 Integration service generates the sample sheet. Run/Analysis configuration is imported manually into NextSeq 1000/2000 instrument via a sample sheet to start the run. The downstream secondary analysis is done using the onboard DRAGEN module.

Hybrid Mode

NextSeq 1000/2000 Integration service sends the run/analysis configuration to Illumina Analytics Platform (IAP). Run/Analysis configuration is downloaded to NextSeq 1000/2000 instrument via IAP to start the run. The downstream secondary analysis is done using the onboard DRAGEN module.

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Cloud Mode

NextSeq 1000/2000 Integration service sends the run/analysis configuration to Illumina Analytics Platform (IAP). Run/Analysis configuration is downloaded to NextSeq 1000/2000 instrument via IAP to start the run. The downstream secondary analysis is performed in the cloud.

Analysis Workflow Details

The analysis workflow selection determines if the sample is advanced to Demultiplexing (NextSeq 1000/2000 Sequencing v1.0) step or completed at AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.0) step.

GenerateFASTQ

After the sequencing run completes, NextSeq 1000/2000 integration service automatically completes the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.0) step. Samples are advanced to the Demultiplexing (NextSeq 1000/2000 Sequencing v1.0) step.

None

None is selected to skip Demultiplexing (NextSeq 1000/2000 Sequencing v1.0) step. NextSeq 1000/2000 integration service automatically completes the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.0) and removes the samples from the workflow.

Step 3: AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.0) Step

> Do not add samples to the Ice Bucket or start or complete the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.0) step. The integration does this action automatically.

This step is fully automated.

The integration starts the step automatically and data from the run is parsed back to Clarity LIMS. No user interaction is required. However, you can open and review the various stages of the step in Clarity LIMS. Do not perform any action when reviewing the data.

Review Run Data

Read summary metrics are recorded for the library pool in the Step Details section and the Sample Details table.

Step Details Section

Values are populated in the following step UDFs/master step fields:

Run Name	Run Status	Output Folder
Current Read	Current Cycle	Secondary Analysis Workflow
Flow Cell ID	Flow Cell Lot Number	Reagent Cartridge ID
Reagent Cartridge Lot Number	Instrument Platform	Instrument Type
Instrument ID	Instrument Control Software Version	RTA Version
Sequencing Log		

Sample Details Table

The summary metrics (per run level) populate in the following UDFs/global fields.

- % Bases >=Q30 R1
- % Bases >=Q30 R2
- % Error Rate R1
- % Error Rate R2
- Yield (Gb) R1
- Yield (Gb) R2
- Reads PF R1
- Reads PF R2
- %PF R1
- %PF R2
- % Aligned R1
- % Aligned R2
- % Phasing R1
- % Phasing R2
- % Prephasing R1
- % Prephasing R2
- Intensity Cycle 1 R1
- Intensity Cycle 1 R2
- Cluster Density R1
- Cluster Density R2

At the end of this step, the pool of samples undergoes either one of the following two actions:

1. If the Analysis Workflow UDF in Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.0) step is set to GenerateFASTQ, the pool automatically advances to and is queued for the Demultiplexing (NextSeq 1000/2000 Sequencing v1.0) step.
2. If the Analysis Workflow UDF in Load To Reagent Cartridge (NextSeq 1000/2000 Sequencing v1.0) step is set to None, the pool automatically completes AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.0) step and gets removed from the workflow.

Step 4: Demultiplexing (NextSeq 1000/2000 Sequencing v1.0)

Do not add samples to the Ice Bucket or start the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.0) step. The integration does this action automatically.

This step is semiautomated.

The integration starts the step automatically and demultiplexing data from the GenerateFASTQ secondary analysis is parsed back to Clarity LIMS. Review the data parsed and assign QC, depending on the criteria set, and complete the step.

In the Record Details screen:

1. Review demultiplexing data.
   - Demultiplexing metrics are recorded for the library pool in the Sample Details table. Metrics include columns for # Reads, # Perfect Index Reads, # One Mismatch Index Reads, and # of >= Q30 Bases (PF).
   - The “Demultiplex_stats.csv” file generated by the Dragen onboard or Cloud analysis module is stored under Files section.
2. Assign QC flags to all the individual sample. There are two ways of doing this step.
   a. Manually assign QC flags through the QC column in Sample Details table.
   b. Automatically assign QC flag by running Assign QC flags automation. This option is for scenarios where a huge number of libraries are involved.

   In the Step Details section, the following step UDF fields are visible. (N is the number of criteria. You can use one or more criteria.)
   - Criteria N - Source Data Field—Select from preset options eg # Reads.
   - Criteria N - Operator—Select from preset options, for example, >= (greater than or equal to).
   - Criteria N - Threshold Value—Enter the desired threshold value.
3. After filling up the criteria fields, select Assign QC flags. This selection triggers the automation script, which loops through each library in the pool and apply QC flag base on the criteria set previously.
   This automation also generates an AssignQC Result file under Files section.
4. Select Next Steps.
   On the Assign Next Steps screen, the Next Step field for all samples is prepopulated with Mark protocol as complete.

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5. Select Finish Step.

At this point, the whole NextSeq 1000/2000 Integration workflow is fully validated.

How The Integration Works

Refer to *NextSeq 1000/2000 Integration v1.0 User and Configuration Guide*.

Troubleshooting

If an automation trigger does not appear to run its corresponding scripts, see *Troubleshooting EPP/Automation* in the Clarity LIMS documentation.

If an error occurs that does not provide direction on how to proceed, complete the following steps:

1. Confirm the version of the installed Illumina NextSeq 1000/2000 Integration Package by running the following command on the Clarity LIMS server command line:

```
rpm -qa | grep SIS
```

2. If the error is related to the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.0) or Demultiplexing (NextSeq 1000/2000 Sequencing v1.0) step, review the log file information.

If the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.0) or Demultiplexing (NextSeq 1000/2000 Sequencing v1.0) steps starts, but it does not complete:

1. Log in to Clarity LIMS using the default user account and use one of the following methods to open the step in Clarity LIMS:
   - Method 1: In Lab View, find the step in the Recent Activities pane.
   - Method 2: Search for the step in Clarity LIMS using reagent cartridge barcode as the search term.
2. On the Record Details screen, the Sequencing Log multiline text field contains logging information.
   - If you are unable to reach the Record Details screen, or if the Sequencing Log field does not contain enough information to resolve the issue, review the `NextSeq1k2k.log` file.
3. Contact the Clarity LIMS support team, supplying the relevant information from the troubleshooting steps already performed.

Logging

In addition to updating the Sequencing Log or Log multiline text field on the AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.0) step or Demultiplexing (NextSeq 1000/2000 Sequencing v1.0) step, the SIS Integration Service writes detailed log files to `/opt/gls/clarity/extensions/SIS/v1/SISServices/NextSeq1k2k.log` and `/opt/gls/clarity/extensions/SIS/v1/SISServices/NextSeq1k2k.error.log`.

If the messages are related to a sequencing run found on either one of the two previous steps, all the log messages include the Reagent Cartridge barcode. In the Clarity LIMS workflows, these barcodes are recorded as container names.

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There is another log file, `/opt/gls/clarity/extensions/SIS/v1/SISServices/UnknownEvents.log`, which logs down other run update events. These events were received but never processed as they are redundant or not linked to any sequencing run.

All the failed events are also logged in `/opt/gls/clarity/extensions/SIS/v1/SISServices/FailedMessage` folder. In this folder, failed events that are specific to a reagent cartridge are logged in individual log file. The file is named after the reagent cartridge container.

To output all the messages that are relevant to a specific sequencing run quickly, enter the following command in the Clarity LIMS server command line:

```
# Please replace the <Reagent Cartridge Barcode> with the desired value eg
EC0000448-EC10
```

```
grep <Reagent Cartridge Barcode>
/opt/gls/clarity/extensions/SIS/v1/SISServices/NextSeq1k2k.log
```

NextSeq1k2k.log

This log file contains messages of all the actions taken by SIS Integration Service in processing the NextSeq 1000/2000 sequencing run update events received and updating Clarity LIMS steps.

The following example shows the output of the previous command.

```
2020-03-18 22:48:41,437 INFO [DefaultQuartzScheduler_Worker-4] [EC0000448-EC10]
NextSeq1k2k (NextSeq1k2kIntegrationChain) - message 0cfe0c10-785e-430c-ad4d-
5220f44f4895 start processing...
```

```
2020-03-18 22:48:41,438 INFO [DefaultQuartzScheduler_Worker-4] [EC0000448-EC10]
NextSeq1k2k (NextSeq1k2kIntegrationChain) - raw body:
{"gdsFolderPath":"/Runs/200318_MP2-13_62_14FD1COMC_
r.dDdoSW4ec0KNtsu3T2yShg","gdsVolumeName":"bssh.61dc14e8d8233bd1bea366f9c8388254
","reagentBarcode":"EC0000448-EC10","v1pre3Id":"194298116","dateModified":"
```

```
2020-03-18T22:48:26.0144696Z","acl":["wid:61dc14e8-d823-3bd1-bea3-
66f9c8388254"],"flowcellBarcode":"14FD1COMC","sampleSheetName":"SampleSheet.csv"
,"apiUrl":"https://api.basespace.illumina.com/v2/runs/r.dDdoSW4ec0KNtsu3T2yShg",
"name":"200318_MP2-13_62_
14FD1COMC","id":"r.dDdoSW4ec0KNtsu3T2yShg","instrumentRunId":"200318_MP2-13_62_
14FD1COMC","status":"Running"}
```

```
2020-03-18 22:48:46,217 INFO [DefaultQuartzScheduler_Worker-4] [EC0000448-EC10]
NextSeq1k2k (RunEventHandler) - Handling sequencing started message.....
```

```
2020-03-18 22:48:46,423 INFO [DefaultQuartzScheduler_Worker-4] [EC0000448-EC10]
NextSeq1k2k (ClarityApiHelper) - Founded steps for process type AUTOMATED -
Sequencing Run (NextSeq 1000/2000 Sequencing v1.0)
```

```
2020-03-18 22:48:46,424 INFO [DefaultQuartzScheduler_Worker-4] [EC0000448-EC10]
NextSeq1k2k (SequencingRunStartedEventHandler) - Searching for container with
name 'EC0000448-EC10'
```


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```
2020-03-18 22:48:47,323 INFO [DefaultQuartzScheduler_Worker-4] [EC0000448-EC10]
NextSeq1k2k (SequencingRunStartedEventHandler) - Found container '27-2206' with
name 'EC0000448-EC10'
2020-03-18 22:48:48,944 INFO [DefaultQuartzScheduler_Worker-4] [EC0000448-EC10]
NextSeq1k2k (RunEventHandler) - Updating reagent lot 'EC0000448-EC10.20440525'
for kit 'NextSeq 1000/2000 Reagent Cartridge'. Status 'ACTIVE' and expiry date
'2021-03-03'
2020-03-18 22:48:49,145 INFO [DefaultQuartzScheduler_Worker-4] [EC0000448-EC10]
NextSeq1k2k (SequencingRunStartedEventHandler) - Starting step for 1 samples:
'2-14865'.
2020-03-18 22:48:51,759 INFO [DefaultQuartzScheduler_Worker-4] [EC0000448-EC10]
NextSeq1k2k (SequencingRunStartedEventHandler) - Successfully started
https://qa7clarityintegration01.cavc.illumina.com/api/v2/steps/24-6478 step.
2020-03-18 22:48:51,760 INFO [DefaultQuartzScheduler_Worker-4] [EC0000448-EC10]
NextSeq1k2k (RunEventHandler) - Sequencing run STARTED
2020-03-18 22:48:52,013 INFO [DefaultQuartzScheduler_Worker-4] [EC0000448-EC10]
NextSeq1k2k (RunEventHandler) - Updating details on step
https://qa7clarityintegration01.cavc.illumina.com/api/v2/steps/24-6478/details.
2020-03-18 22:48:52,254 INFO [DefaultQuartzScheduler_Worker-4] [EC0000448-EC10]
NextSeq1k2k (RunEventHandler) - Updated field 'Run Name' value to 'MP2-13_TLP_
LongRead_CLIMS'
2020-03-18 22:48:52,254 INFO [DefaultQuartzScheduler_Worker-4] [EC0000448-EC10]
NextSeq1k2k (RunEventHandler) - Updated field 'Output Folder' value to
'/mnt/Vega/SequencingRuns/200318_MP2-13_62_14FD1COMC'
```

NextSeq1k2k.error.log

This log file is the summary of all the error messages encountered for all the run update event processing.

The following example shows the output of the previous command.

```
2020-03-15 09:53:49,582 ERROR [DefaultQuartzScheduler_Worker-6] [EC0000109-EC10]
NextSeq1k2k (NextSeq1k2kIntegrationChain) - No 'AUTOMATED - Sequencing Run
(NextSeq 1000/2000 Sequencing v1.0)' steps with Reagent Cartridge ID ==
'EC0000109-EC10' is started.
2020-03-15 09:53:49,583 ERROR [DefaultQuartzScheduler_Worker-6] [EC0000109-EC10]
NextSeq1k2k (NextSeq1k2kIntegrationChain) - message 2579f8db-6b17-4441-bfeb-
68d33e5803a4 failed to process: {"gdsFolderPath":"/Runs/200313_MP2-14_73_
AAAC5VNM5_
r.8bVi9Cs180G8AU7OKW1cmA","gdsVolumeName":"bssh.61dc14e8d8233bd1bea366f9c8388254
","reagentBarcode":"EC0000109-EC10","v1pre3Id":"194204104","dateModified":"
```

2020-03-15T09:53:37.9806396Z","acl":["wid:61dc14e8-d823-3bd1-bea3-66f9c8388254"],"flowcellBarcode":"AAAC5VNM5","sampleSheetName":"SampleSheet.csv","apiUrl":"https://api.basespace.illumina.com/v2/runs/r.8bVi9Cs180G8AU7OKW1cmA","name":"200313_MP2-14_73_AAAC5VNM5","id":"r.8bVi9Cs180G8AU7OKW1cmA","instrumentRunId":"200313_MP2-14_73_AAAC5VNM5","status":"Uploading"}

UnknownEvents.log

This log file stores all other run update events that Clarity LIMS receives but are never processed because no corresponding sequencing run is found. If the prior two log files did not give any insights to the error, do a quick search on this log file to see if the run update event for the sequencing run is present in this file.

The following example shows the output of the previous command.

2020-03-12 17:34:42,862 INFO [DefaultQuartzScheduler_Worker-9] [2f223ffc-3916-4d12-a217-fe7633bac328] UnknownEvents (IntegrationChain) - {"gdsFolderPath":"/Runs/200311_MP2-18_63_12BH1COMC_r.fnzlB3ZU80SEU35dBWiYrA","gdsVolumeName":"bssh.61dc14e8d8233bd1bea366f9c8388254","reagentBarcode":"EC0000130-EC10","v1pre3Id":"194113926","dateModified":"
2020-03-12T17:34:40.4492111Z","acl":["wid:61dc14e8-d823-3bd1-bea3-66f9c8388254"],"flowcellBarcode":"12BH1COMC","sampleSheetName":"SampleSheet.csv","apiUrl":"https://api.basespace.illumina.com/v2/runs/r.fnzlB3ZU80SEU35dBWiYrA","name":"200311_MP2-18_63_12BH1COMC","id":"r.fnzlB3ZU80SEU35dBWiYrA","instrumentRunId":"200311_MP2-18_63_12BH1COMC","status":"Uploading"}
2020-03-12 17:35:50,147 INFO [DefaultQuartzScheduler_Worker-2] [dc15da71-80c8-4461-a4c0-d19330a47062] UnknownEvents (IntegrationChain) - {"gdsFolderPath":"/Runs/200311_MP2-18_63_12BH1COMC_r.fnzlB3ZU80SEU35dBWiYrA","gdsVolumeName":"bssh.61dc14e8d8233bd1bea366f9c8388254","reagentBarcode":"EC0000130-EC10","v1pre3Id":"194113926","dateModified":"
2020-03-12T17:35:45.7820780Z","acl":["wid:61dc14e8-d823-3bd1-bea3-66f9c8388254"],"flowcellBarcode":"12BH1COMC","sampleSheetName":"SampleSheet.csv","apiUrl":"https://api.basespace.illumina.com/v2/runs/r.fnzlB3ZU80SEU35dBWiYrA","name":"200311_MP2-18_63_12BH1COMC","id":"r.fnzlB3ZU80SEU35dBWiYrA","instrumentRunId":"200311_MP2-18_63_12BH1COMC","status":"PendingAnalysis"}
2020-03-12 17:37:06,181 INFO [DefaultQuartzScheduler_Worker-6] [8c932111-96b0-4ef7-a94b-2efe35ecede2] UnknownEvents (IntegrationChain) - {"gdsFolderPath":"/Runs/200311_MP2-18_63_12BH1COMC_r.fnzlB3ZU80SEU35dBWiYrA","gdsVolumeName":"bssh.61dc14e8d8233bd1bea366f9c8388254","reagentBarcode":"EC0000130-EC10","v1pre3Id":"194113926","dateModified":"

```
2020-03-12T17:37:05.4457500Z","acl":["wid:61dc14e8-d823-3bd1-bea3-
66f9c8388254"],"flowcellBarcode":"12BH1COMC","sampleSheetName":"SampleSheet.csv"
,"apiUrl":"https://api.basespace.illumina.com/v2/runs/r.fnzlB3ZU80SEU35dBWiYrA",
"name":"200311_MP2-18_63_
12BH1COMC","id":"r.fnzlB3ZU80SEU35dBWiYrA","instrumentRunId":"200311_MP2-18_63_
12BH1COMC","status":"Analyzing"}
2020-03-12 17:53:56,782 INFO [DefaultQuartzScheduler_Worker-7] [a7a4d79a-bbae-
4e76-ad71-0c4d0dced79e] UnknownEvents (IntegrationChain) -
{"gdsFolderPath":"/Runs/200311_MP2-18_63_12BH1COMC_
r.fnzlB3ZU80SEU35dBWiYrA","gdsVolumeName":"bssh.61dc14e8d8233bd1bea366f9c8388254
","reagentBarcode":"EC0000130-EC10","v1pre3Id":"194113926","dateModified":"
2020-03-12T17:53:56.1491410Z","acl":["wid:61dc14e8-d823-3bd1-bea3-
66f9c8388254"],"flowcellBarcode":"12BH1COMC","sampleSheetName":"SampleSheet.csv"
,"apiUrl":"https://api.basespace.illumina.com/v2/runs/r.fnzlB3ZU80SEU35dBWiYrA",
"name":"200311_MP2-18_63_
12BH1COMC","id":"r.fnzlB3ZU80SEU35dBWiYrA","instrumentRunId":"200311_MP2-18_63_
12BH1COMC","status":"Complete"}
```


Reprocess a Run Status Update Event Message

In AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v1.0) or Demultiplexing (NextSeq 1000/2000 Sequencing v1.0) step, some cases might result in failed processing of a run status update event by SIS integration service. In this scenario, troubleshoot, identify the problem, and fix it. After fixing the issue, reprocess the same run status update event message, so the samples can complete the workflow.

Here are the steps to reprocess a message:

1. From `NextSeq1k2k.log` log file or `/opt/gls/clarity/extensions/SIS/v1/SISServices / FailedMessage` folder, find the message body to reprocess. For example:


```
{"gdsFolderPath":"/Runs/200312_SimulatedInstrument_2_19131CHMC_
r.iUN56ZaHckipNpoXjxThiQ","gdsVolumeName":"bssh.61dc14e8d8233bd1bea366f9c8388254
","reagentBarcode":"YS1234567-YS01","v1pre3Id":"194179067","dateModified":"
2020-03-12T21:48:16.9334806Z","acl":["wid:61dc14e8-d823-3bd1-bea3-
66f9c8388254"],"flowcellBarcode":"19131CHMC","sampleSheetName":"SampleSheet.csv"
,"apiUrl":"https://api.basespace.illumina.com/v2/runs/r.iUN56ZaHckipNpoXjxThiQ",
"name":"200312_SimulatedInstrument_2_
19131CHMC","id":"r.iUN56ZaHckipNpoXjxThiQ","instrumentRunId":"200312_
SimulatedInstrument_2_19131CHMC","status":"Running"}
```


2. Execute as root:

```
java -jar /opt/gls/clarity/extensions/SIS/v1/sis-core-automation.jar
script:push_to_sqs -m '{yourMessage}'
```

3. You should see the following message:

```
Sending message is successful with message id {theMessageIdSendSuccessfully}
```

4. Then, if it is reprocessed successfully by SIS integration service, you should see the message in `NextSeq1k2k.log`.

NextSeq 1000/2000 Integration v1.0.5 Release Notes

The NextSeq 1000/2000 Integration v1.0.5 has the following compatibilities:

- Clarity LIMS v5.3 only
- NGS Extensions Package v5.19 and later
- NextSeq 1000/2000 Control Software v1.1 and v1.2

Features to Note for this Release

This release of Clarity LIMS integration package provides:

- Support for the P3 flow cell.
- Support for Illumina Analytics Platform and BlueBee integration with enhanced sample sheet.
- Bug fixes.

Defect Fixed

- Fixed typographical error in the installation script.

NextSeq 500/550 Integrations

The documents in this section support the Illumina NextSeq 500/550 Integration.

NextSeq 500/550 v2.3 Configuration and User Interaction

The Illumina NextSeq 500/550 Integration Package v2.3 supports the integration of Clarity LIMS to Illumina NextSeq 500 and 550 sequencing systems.

The integration allows for automated tracking of an Illumina sequencing run in Clarity LIMS, which includes tracking instrument run status, generating run report, and capturing and parsing run statistics. In addition, this integration provides automated generation of a sample sheet file for use with bcl2fastq2 v2.20.0 analysis software.

This document describes the integration between Clarity LIMS and the NextSeq system. It includes information about protocols and automations, configuration options, installed components, and rules and constraints.

For instructions on validating and troubleshooting the NextSeq 500/550 Integration Package, refer to *NextSeq 500/550 Integration v2.3 Validation and Troubleshooting on page 1824*.

Integration Overview

The NextSeq 500/550 Integration Package v2.3 includes the following items:

- Automated generation of a sample sheet for use with bcl2fastq2 v2.20.0 analysis software.
- Automated tracking of the NextSeq sequencing run and parsing of run statistics into Clarity LIMS, including:
  - Run status and metrics of sequencing run
  - Sequencing run parameters
  - Real-Time Analysis v2 (RTA2) run directory location and other run-specific information

Prerequisites and Assumptions

It is assumed that samples enter the NextSeq 500/550 Sequencing v1.1 workflow as normalized libraries and have reagent labels attached.

That is, before they are assigned to the workflow:

- Samples have been accessioned into Clarity LIMS.
- Samples have been run through QC and library prep.
- Samples have been normalized, and the value is captured in a field called Normalized Molarity (nM).

For more information on sample accessioning, refer to *Sample Accessioning* and *Upload and Modify Samples* in the Getting Started section of the Clarity LIMS Core Functionality documentation.

You can assign samples to workflows automatically, using a routing script, or manually—from the Projects & Samples dashboard. Refer to *Assign and Process Samples* in the Clarity LIMS Core Functionality documentation.

Workflows, Protocols, and Steps

NextSeq Integration Package v2.3 includes the NextSeq 500/550 Sequencing v1.1 workflow, which contains a single protocol of the same name.

The NextSeq 500/550 Sequencing v1.1 protocol includes the following steps:

- Library Pooling (NextSeq 500/550 v1.1)
- Denature & Dilute (NextSeq 500/550 v1.1)
- NextSeq 500/550 Run (NextSeq 500/550 v1.1)

Step Automations

The following table lists the automations configured on the NextSeq 500/550 Sequencing v1.1 steps. Automation details are provided in the configuration details sections following the table. For details on the validation workflow, refer to *NextSeq 500/550 Integration v2.3 Validation and Troubleshooting* on page 1824.

Table 371 NextSeq 500/550 Sequencing System v1.1 Protocol Step Automation

Step	Automation
1. Library Pooling (NextSeq 500/550 v1.1)	• Set Next Step - Advance
2. Denature and Dilute (NextSeq 500/550 v1.1)	• Generate bcl2fastq2 NextSeq 500/550 SampleSheet • Set Next Step - Advance
3. NextSeq 500/550 Run (NextSeq 500/550 v1.1)	• Set Next Step - Advance

Step 1: Library Pooling (NextSeq 500/550 v1.1)

The Library Pooling (NextSeq 500/550 v1.1) step is derived from the Library Pooling v1.0 master step. Libraries are placed into pools manually.

Set Next Step - Advance Automation

This automation is automatically triggered on exit of the Record Details screen. The automation advances samples to the next step in the protocol.

The default command line is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-t false \
-h false \
-exp 'nextStep = ::ADVANCE::' \
-log {compoundOutputFileLuid0}"
```

Master Step Fields

This field is configured on the Library Pooling (NextSeq 500/550 v1.1) step and displays on the Record Details screen at run time.

The following table shows field configuration details.

Table 372 Library Pooling (NextSeq 500/550 v1.1) Master Step Field Configuration

Field Name	Field Type	Field Constraints/Options
Comment	Multiline Text	

Global Fields

The following table lists the global custom fields configured to display on the Queue and Ice Bucket screens of the Library Pooling (NextSeq 500/550 v1.1) step. Most of these fields show in the expanded view only.

Table 373 Global Custom Field Configuration for Library Pooling (NextSeq 500/550 v1.1)

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Configured on submitted sample:			

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Application	Text Dropdown	Custom Entries	Presets • TruSeq mRNA sequencing • TruSeq DNA sequencing (large genome de novo) • TruSeq DNA sequencing (large genome re-seq) • TruSeq DNA sequencing (small genome de novo) • TruSeq DNA sequencing (small genome re-seq) • Nextera DNA sequencing • TruSeq Custom Amplicon sequencing • ChIP-sequencing • Exome sequencing • Mate pair sequencing • Small RNA sequencing
Pooling	Text Dropdown	Custom Entries	Presets • Yes • No
Read Length	Text		Not applicable
Sequencing Coverage	Text		Not applicable

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Sequencing Method	Text Dropdown	Custom Entries	Presets • Single Read • Paired End Read • Indexed Single read • Indexed Paired End Read
Configured on derived sample:			
Normalized Molarity (nM)	Numeric		Decimal places displayed = 2

User Interaction

1. On the Queue screen, add the samples to the Ice Bucket.
2. In the Add Control Samples panel, add the PhiX v3 control sample to the Ice Bucket.
3. Select View Ice Bucket.
4. On the Ice Bucket screen, select Begin Work.
5. On the Pool Samples screen, create a pool of samples by dragging samples into the Pool Creator. Name the pool or accept the default name (Pool #1).
6. Select Place Samples.
7. On the Placement screen, select the pool in the Samples to be Placed area on the left and drag it to the container on the right.
8. Select Record Details.
9. On the Record Details screen, select Next Steps.
10. On the Assign Next Steps screen, make sure the next step is set to Denature and Dilute (NextSeq 500/500 v1.1).
11. Select Finish Step.

Step 2: Denature and Dilute (NextSeq 500/550 v1.1)

In this step, pooled libraries are denatured and diluted and placed into the reagent cartridge that is loaded into the NextSeq instrument.

Generate bcl2fastq2 NextSeq SampleSheet Automation

This automation is triggered by a button on the Record Details screen.

This automation generates the sample sheet and attaches it to the step. For details, see the following section on sample sheet generation.

The default command line is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/DriverFileGenerator.jar -i {processURI:v2} -u {username} -p {password} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/{udf:Samplesheet Template} \
-o {compoundOutputFileLuid1}.csv \
-q true \
-destLIMSID {compoundOutputFileLuid1} \
-l {compoundOutputFileLuid2}"
```

Set Next Step - Advance Automation

This automation is automatically triggered on exit of the Record Details screen.

This automation advances samples to the next step in the protocol.

The default command line is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-t false \
-h false \
-exp 'nextStep = ::ADVANCE::' \
-log {compoundOutputFileLuid3}"
```

Master Step Fields

There are 10 fields configured on the Denature and Dilute (NextSeq 500/550 v1.0) step. At run time, these fields display on the Record Details screen, in the Step Data table. The fields are manually populated. Their values are used to generate the sample sheet.

The following table lists field configuration details.

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Table 374 Denature and Dilute (NextSeq 500/550 v1.1) Master Step Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Adapter	Text		Not applicable
Adapter Read 2	Text		Not applicable
Experiment Name	Text		Not applicable
Mask Adapter	Text		Not applicable
Mask Adapter Read 2	Text		Not applicable
Read 1 Cycles	Numeric Dropdown	Required Field Custom Entries	Presets • 251 (Default) • 151 • 101 • 76 • 51 Range = 0–1000 Decimal places displayed = 0
Read 2 Cycles	Numeric Dropdown	Custom Entries	Presets • 251 • 151 • 101 • 76 • 51 Range = 0–1000 Decimal places displayed = 0
SampleSheet Template	Text Dropdown		Presets • BCL2FASTQ_Reverse_Complement_Samplesheet.csv (default) • BCL2FASTQ_Samplesheet.csv

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Workflow	Text Dropdown	Required Field	Presets • Assembly • Custom Amplicon • Enrichment • GenerateFASTQ • LibraryQC • Metagenomics • PCR Amplicon • Resequencing

Groups of Defaults

The following group of defaults are configured for Denature and Dilute (NextSeq 500/550 v1.1) master step custom fields:

- Resequencing
  - Read 1 Cycles: 251
  - Workflow: Resequencing
- Custom Amplicon
  - Read 1 Cycles: 251
  - Workflow: Custom Amplicon
- PCR Amplicon
  - Read 1 Cycles: 251
  - Workflow: PCR Amplicon
- Enrichment
  - Read 1 Cycles: 251
  - Workflow: Enrichment
- Library QC
  - Read 1 Cycles: 251
  - Workflow: LibraryQC
- Metagenomics
  - Read 1 Cycles: 251
  - Workflow: Metagenomics
- GenerateFastQ

  - Read 1 Cycles: 251
  - Workflow: GenerateFastQ
- Assembly
  - Read 1 Cycles: 251
  - Workflow: Assembly

Global Fields

The following table lists the global fields that are configured to display on the Queue, Ice Bucket, and Record Details screens of the Denature and Dilute (NextSeq 500/550 v1.1) step.

Table 375 Global Custom Field Configuration for Denature and Dilute (NextSeq 500/550 v1.1) Step

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Configured on submitted sample:			
Read Length Displays on Queue & Ice Bucket screens	Text		Not applicable
Sequencing Method Displays on Queue & Ice Bucket screens	Text Dropdown	Custom Entries	Presets • Single Read • Paired End Read • Indexed Single Read • Indexed Paired End Read
Configured on derived sample:			
Final Loading Concentration Displays on Record Details screen	Numeric Dropdown	Required Field Custom Entries	Presets • 225 • 400 Decimal places displayed = 0

Step File Placeholders

Placeholders for the following files are configured on the Record Details screen of the Denature and Dilute (NextSeq 500/550 v1.1) step:

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- Lab Tracking Form (manually generated)
- bcl2fastq SampleSheet (autogenerated)
- bcl2fastq SampleSheet Generation Log (autogenerated)
- Log File (autogenerated)

Table 376 Sample Sheet Files & Placeholders Generated by Denature & Dilute (NextSeq 500/550 v1.1) Step

Item	Description
Lab Tracking Form	This form in Clarity LIMS allows for manually attaching a lab-specific tracking form to the step.
bcl2fastq SampleSheet	This CSV file is automatically generated by Clarity LIMS for use with the bcl2fastq2 v2.20.0 analysis software. It can be opened as a text file or as an MS Excel spreadsheet.
bcl2fastq SampleSheet Generation Log	Automatically generated by Clarity LIMS, this log file captures any errors that Clarity LIMS can encounter when generating the sample sheet.
Log File	Automatically generated by Clarity LIMS, this log file captures the status of the EvaluateDynamicExpression script that is launched by the Set Next Step - Advance automation.

User Interaction

1. On the Queue screen, add the pool to the Ice Bucket and select View Ice Bucket.
2. On the Ice Bucket screen, select Begin Work.
3. On the Placement screen, scan the barcode of the NextSeq reagent cartridge into the NextSeq Reagent Cartridge field. Place the pool of samples into the reagent cartridge.
4. Select Record Details.
5. On the Record Details screen under Reagent Lot Tracking, select the reagent lot used in the step. If necessary, add/activate the lot on the Reagents and Controls screen.
6. Populate the fields in the Step Details table.
   Workflow and Read 1 Cycles are required fields.

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7. In the Sample Details table, enter a Final Loading Concentration value. Select from the two preset options: 225 (for PCR-free workflows) or 400 (for Nano workflows). You can also enter a different value manually.
8. Select Generate bcl2fastq2 NextSeq SampleSheet.
9. Clarity LIMS generates the samplesheet and attaches it and a log file to placeholders in the Files area of the Record Details screen. Download the files and validate their format and content.
10. Select Next Steps.
    On the Assign Next Steps screen, samples are assigned to the NextSeq 500/550 Run (NextSeq 500/550 v1.1) step.
11. Select Finish Step.

Step 3: NextSeq 500/550 Run (NextSeq 500/550 v1.1)

In this step, pooled samples are sequenced on the NextSeq 500/550 instrument and the run metrics are recorded in Clarity LIMS.

Set Next Step - Advance Automation

This automation is automatically triggered on exit of the Record Details screen. The automation advances samples to the next step in the protocol.

The default command line is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-t false \
-h false \
-exp 'nextStep = ::ADVANCE::' \
-log {compoundOutputFileLuid5}"
```

Master Step Fields

There are 16 fields configured on the NextSeq 500/550 Run (NextSeq 500/550 v1.1) step. These fields display on the Record Details screen at run time. Some of the field values must be completed manually, and the remaining fields are automatically populated at the end of the run.

The following table lists field configuration details.

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Table 377 NextSeq 500/550 Run (NextSeq 500/550 v1.1) Master Step Field Configuration

Field Name	Field Type	Filed Constraints/Options	Preset Value/Additional Options and Drop-down Items
Chemistry	Text	Read Only	Not applicable
Comment	Multiline Text		Not applicable
Experiment Name	Text	Read Only	Not applicable
Finish Date	Date	Read Only	Not applicable
Flow Cell ID	Text	Read Only	Not applicable
Index 1 Read Cycles	Numeric	Read Only	Decimal places displayed = 0
Index 2 Read Cycles	Numeric	Read Only	Decimal places displayed = 0
Output Folder	Text	Read Only	Not applicable
PR2 Bottle ID	Text	Read Only	Not applicable
Read 1 Cycles	Numeric	Read Only	Decimal places displayed = 0
Read 2 Cycles	Numeric	Read Only	Decimal places displayed = 0
Reagent Cartridge ID	Text	Read Only	Not applicable
Run ID	Text	Read Only	Not applicable
Run Type	Text	Read Only	Not applicable
Status	Text	Read Only	Not applicable
Workflow	Text	Read Only	Not applicable

Global Fields

There are several sample and measurement global fields configured to display on the Record Details screen of the NextSeq 500/550 Run (NextSeq 500/550 v1.1) step. These fields are populated at the end of the sequencing run.

Step File Placeholders

Placeholders for the following files are configured on the Record Details screen of the NextSeq 500/550 Run (NextSeq 500/550 v1.1) step.

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- Illumina Run Report (autogenerated)
- Link to Run Folder (autogenerated)
- Run Parameters (autogenerated)
- Run Info (autogenerated)
- Lab Tracking Form (manually generated)
- Log File (autogenerated)

User Interaction

1. On the Queue screen, add the pool to the Ice Bucket and select View Ice Bucket.
2. On the Ice Bucket screen, select Begin Work.
   On the Record Details screen, the fields in the table are read-only. After the run completes, the integration automatically:
   - Populates the fields.
   - Attaches files to the Illumina Run Report, Link to Run Folder, Run Parameters, and Run Info placeholders.
   - Populates the fields in the Sample Details table.
3. Select Next Steps.
4. On the Assign Next Steps screen, make sure that the next step is set to Mark protocol as complete.
5. Select Finish Step.

Sample Sheet Generation

Sample sheet generation occurs on the step before the sequencing run Denature and Dilute (NextSeq 500/550 v1.1) step. Samples are placed on the container to be loaded in the instrument. The default configuration provides the Generate bcl2fastq2 NextSeq Samplesheet automation.

The Generate bcl2fastq2 NextSeq Samplesheet automation uses the Template File Generator (`DriverFileGenerator.jar`) and a template file to generate a CSV format file for use with bcl2fastq2 v2.20.0 analysis software. The sample sheet content is determined by the fields that display on the Record Details screen of the step (in the Step Data table) and the values entered into the fields. Templates can be customized to create the sample sheet. If additional columns are required by the lab, then they can be inserted.

Sequencing Results Parsing

The NextSeq 500/550 Run (NextSeq 500/550 v1.1) step records information for the flow cell lanes and generates a report summarizing the results. In addition, run parameters, run info, and a link to the run folder are automatically captured.

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Generated and Captured Files

The following table lists the run information files, reports, placeholders, and links that Clarity LIMS automatically generates or capture during a sequencing run.

Table 378 Run Information Generated or Captured by NextSeq 500/550 Run (NextSeq 500/550 v1.1) Step

Item	Description
Run Info Run Parameters	These XML files are captured automatically by Clarity LIMS from the instrument run folder. They include the key run parameters, many of which are parsed out into key step global custom fields.
Link to Run Folder	Automatically generated by Clarity LIMS, and is a link to the network run folder where the data that was captured from the instrument during the run is stored.
Illumina Run Report	Automatically generated by Clarity LIMS, this report provides key information about the run and the samples on the flow cell. Information includes the flow cell ID, run directory location, and primary analysis metrics for the instrument, summarized per flow cell lane for the entire run, and individual reads if there are paired-end runs. These metrics are compared against the instrument per lane averages, calculated using metrics from the last 5 sequencing runs. Any values outside of 1 standard deviation are highlighted.
Lab Tracking Form	This placeholder in Clarity LIMS allows you to attach a lab-specific tracking form to the step manually.

Metadata

The following list includes the metadata that Clarity LIMS automatically captures from the Illumina sequencing software as part of a sequencing run. This information is gathered from various run result files and events.

- Chemistry

- Experiment Name – entered in software
- Finish Date—Run completion date
- Flow Cell ID
- Index 1 Read Cycles—Intended Index cycles
- Index 2 Read Cycles—Intended Index cycles
- Output Folder—Run folder root
- PR2 Bottle ID
- Reagent Cartridge ID
- Read 1 Cycles
- Read 2 Cycles
- Run ID—The unique run ID
- Run Type
- Status—Current status of the sequencing run on the instrument
- Workflow

If the End Run event contains a date in the format YYYY-MM-DD, Finish Date is set to the date in the event file. If the End Run event does not contain a date or the date is in the wrong format, Finish Date is set to the date when the event file is processed.

Primary Analysis Metrics

The following table lists the Real-Time Analysis v2 (RTA2) primary analysis metrics that Clarity LIMS automatically captures and records, per read, for samples in each flow cell lane. These metrics are captured after run completion and are stored as global custom fields in the Record Details screen Sample Details table. Per read and per lane metrics are viewable by expanding the output.

Table 379 RTA Primary Analysis Metrics Captured by NextSeq 500/550 Run (NextSeq 500/550 v1.0) Step

Per Read Clarity LIMS Field Name (stored on derived sample/analyte input to the step)	Per Lane Clarity LIMS Field Name (stored in measurement placeholders in Record Details screens Sample Details table)
% Aligned R1	% Aligned R1
% Aligned R2	% Aligned R2
% Bases >=Q30 R1	% Bases >=Q30 R1
% Bases >=Q30 R2	% Bases >=Q30 R2

Per Read Clarity LIMS Field Name (stored on derived sample/analyte input to the step)	Per Lane Clarity LIMS Field Name (stored in measurement placeholders in Record Details screens Sample Details table)
% Error Rate R1	% Error Rate R1
% Error Rate R2	% Error Rate R2
	% Phasing R1
	% Phasing R2
	% Prephasing R1
	% Prephasing R2
	%PF R1
	%PF R2
	Cluster Density (K/mm^2) R1
	Cluster Density (K/mm^2) R2
Intensity Cycle 1 R1	Intensity Cycle 1 R1
Intensity Cycle 1 R2	Intensity Cycle 1 R2
	Reads PF (M) R1
	Reads PF (M) R2
Yield PF (Gb) R1	Yield PF (Gb) R1
Yield PF (Gb) R2	Yield PF (Gb) R2

The sequencing service runs on the Clarity LIMS server. The service detects event files that the instrument software (RTA2) produces as the run progresses, which tells the service where to find the run data. As the run data is written out and the End Run event is detected, the data is matched to the step. This matching is based on the reagent cartridge ID that was entered/scanned in the Denature and Dilute (NextSeq 500/550 v1.1) step. Read-only field values on the Record Details screen are populated accordingly. When finished processing the end run event and updating the fields in Clarity LIMS, the sequencing service generates the report and attaches it to the step.

User Interaction

1. Begin the step process and wait until the sequencing service has finished processing the run and the run report is generated and attached.
2. On the Record Details screen, the read-only field values are automatically populated as the instrument runs.
   After the run has completed:
   - The read-only fields are populated.

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- The Illumina Run Report is generated and attached.
- The Link to Run Folder is generated and attached.
- The Run Parameters and Run Info files are attached.

3. [Optional] In the Files section, upload the Lab Tracking Form.

   Do not complete the following step until the Illumina Run Report is attached to the step in Clarity LIMS.

4. Complete the step. The Log File is attached after the next step for samples is assigned by the Next Step, Advance automation.

Components Installed

This integration requires installation of the Illumina Preset Protocols (IPP). For details, refer to *NextSeq 500/550 Integration v2.3 Release Notes* on page 1841.

Scripts and Files Installed

The following table lists the scripts and files installed in the Illumina NextSeq 500/550 Integration Package v2.3 RPM.

Table 380 Illumina NextSeq 500/550 Integration Package v2.3 Scripts and Files Installed

Files Installed	Location and Description
`configure_extensions_nextseq_sequencingservice-v2.sh`	`/opt/gls/clarity/config/` Script that installs the service properties in the database.
`log4j2.xml`	`/opt/gls/clarity/extensions/Illumina_NextSeq/v2/SequencingService/conf` File containing the settings for the sequencing jar logging.
`nextseq-sequencing-v2.jar`	`/opt/gls/clarity/extensions/Illumina_NextSeq/v2/SequencingService` Jar file containing API-based Clarity LIMS extensions used for capturing run results and report generation.
InterOp libraries	`opt/gls/clarity/extensions/Illumina_NextSeq/v2/lib` Shared library for parsing InterOp data files.

Files Installed	Location and Description
Installed from IPP • `BCL2FASTQ_Reverse_Complement_Samplesheet.csv` • `BCL2FASTQ_Samplesheet.csv`	`/opt/gls/clarity/extensions/conf/driverfiletemplates` Template file used for file generation. In this integration, the reverse complement template is used by default.

Database Properties Installed

The following table lists the database properties installed with Illumina NextSeq 500/550 Integration Package v2.3.

Additional properties, each with the 99 suffix appended to their name, are also installed and intended for use by the Clarity LIMS Support team in automated validation tests. Those properties are not listed in the table.

Sequencing runs are matched using the reagent cartridge ID and the sequencing steps base name. The base name is NextSeq 500/550 Run (NextSeq 500/550 v1.1). Do not change the base name. The name is expected by the sequencing service that captures instrument run results. The base name is stored in the sequenceProcessBaseName database property. If this name is changed without the database property being updated, the 'reagent cartridge ID <-> sequencing step base name' matching system fails. If necessary, modify the step name by editing or adding text after the base name portion, as it is not used in the matching system. For example, change NextSeq 500/550 Run (NextSeq 500/550 v1.1) to NextSeq 500/550 Run (NextSeq 500/550 v1.1) v2.

Table 381 Database Properties Installed with the Illumina NextSeq 500/550 Integration Package

Property	Description	Default Value
nextseq.v2.seqservice.sequenceProcessBaseName (Installed from the IPP)	Sequencing process type / master step base display name. Partial matching is used to look up the process type / master step.	NextSeq 500/550 Run (NextSeq 500/550 v1.1)

Property	Description	Default Value
nextseq.v2.seqservice.eventFileDirectory.1	A network location monitored for event files (eg, `/mnt/illumina/gls_events/`)	/mnt/gls_events
nextseq.v2.seqservice.netPathPrefixSearch.1[1]	The network directory prefix contained in the event file - most likely in Windows format.	\\nas\network\run_data
nextseq.v2.seqservice.netPathPrefixReplace.1	The mapped network directory mount name on the server used to access the run data directory (eg, `/mnt/network/data`)	/mnt/run_data
nextseq.v2.seqservice.eventFileDirectorySuffixes	A list of eventFileDirectory path entries to monitor for event files. The value is one or more comma-separated integers.	99 Configured on install to point to 1.
nextseq.v2.seqservice.netPathPrefixSearchReplaceSuffixes	A list of netPathPrefix search and replace entries for transforming Windows to Linux network paths. The value is one or more comma-separated integers.	99 Configured on install to point to 1.

Property	Description	Default Value
nextseq.v2.seqservice.runReportViewsVersion	The current version of the Run Report views in the database. The value 0 represents the state before the views are created. This property is automatically updated by the run report.	0
nextseq.v2.seqservice.ignoreUnmatchedContainerIds	A flag indicating if event files that cannot be matched to reagent cartridges in Clarity LIMS should be archived after a certain time (true), or continually reprocessed (false).	false Note: To prevent the gls_ events file directory from becoming cluttered, it is recommended that the value of this property is set to true.
nextseq.v2.seqservice.ignoreUnmatchedContainerIdsWaitDays	The number of days between when the event is created and the event file is archived.	14
nextseq.v2.seqservice.synchronizationPeriod	Invocation period in seconds.	60

[1]It is possible to configure support for multiple, identical seqservice.netPathPrefixSearch property values.

Reagent Categories/Label Groups Installed

Reagent categories/label groups are installed with the IPP workflow configuration slices.

The NextSeq Reagent Kit is included in the NextSeq Integration.

Control Types Installed

The PhiX v3 control type is included in the NextSeq Integration.

Container Types Installed

The NextSeq Reagent Cartridge container type is included in the NextSeq Integration.

All one-dimensional container types with both numeric rows and numeric columns are supported.

Instrument Integration

To make sure that the Illumina instrument warranty remains valid, the instrument integration must be performed and maintained by the Clarity LIMS Support team. To perform this integration, the Support team requires remote access to the instrument while it is idle.

To configure the Illumina instrument for use with the Illumina NextSeq Integration, the Support team:

1. Creates a directory on the local computer to hold the batch files. These batch files write event files to the network attached storage (NAS) shares.
2. Creates a directory on the NAS to hold the event files.
3. Modifies Illumina software configuration files to call the batch files that create the event files.
4. Updates sequencing service default database properties to match the specifics of the installation.

Rules and Constraints

This integration operates with the following constraints:

- The reagent cartridge ID must be unique. Avoid multiple reagent cartridge containers in the system with identical names.
- The reagent cartridge ID must be scanned as the reagent cartridge Container Name on the Denature and Dilute (NextSeq 500/550 v1.1) step.

NextSeq 500/550 Integration v2.3 Validation and Troubleshooting

This guide explains how to validate the installation of the Illumina NextSeq 500/550 Integration Package v2.3.

The validation process involves:

- Validating the creation of event files.
- Running samples through the Library Prep Validation v2.3.1 workflow. This workflow is installed by Illumina Preset Protocols (IPP) v2.6 and contains a single-step protocol that models the library prep required to produce normalized libraries. At the end of the step, the normalized libraries are automatically advanced to the NextSeq 500/550 Sequencing v1.1 workflow.

- Running normalized libraries through the NextSeq 500/550 Sequencing v1.1 workflow. This validates the automated generation of a sample sheet for use with bcl2fastq2 v2.20.0 analysis software. It also validates the automated tracking of the NextSeq sequencing run and parsing of run statistics into Clarity LIMS, including:
  - Run status and metrics of sequencing run
  - Sequencing run parameters
  - Real Time Analysis v2 (RTA2) run directory location and other run specific information

Activate Workflow, Create Project, Add and Assign Samples

The following steps set up Clarity LIMS in preparation for running samples through the Library Prep Validation v2.3.1 and NextSeq 500/550 Sequencing v1.1 workflows.

1. Activate the Workflows

In the Clarity LIMS configuration area, activate the Library Prep Validation v2.3.1 and NextSeq 500/550 Sequencing v1.1 workflows.

2. Create a Project, Add Samples

1. On the Projects and Samples screen, create a project and add samples to it.
2. Assign your samples to the Library Prep Validation v2.3.1 workflow.

Library Prep Validation v2.3.1 Protocol

This single-step protocol models the library prep required to produce normalized libraries that are ready for the NextSeq 500/550 Sequencing v1.1 workflow.

Run Library Prep Validation v2.3.1 Step

1. In Lab View, locate the Library Prep Validation v2.3.1 protocol. You will see your samples queued for the Library Prep Validation v2.3.1 step.
2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Placement screen, move the samples as follows.
   a. In the Placed Samples area on the right, in the container field, scan or enter the barcode of the destination container.
   b. Select the samples from the Samples to be Placed area on the left. Drag them over to the container wells on the right.
   c. Select Add Labels.
5. On the Add Labels screen, assign the labels as follows.

a. Select labels on the left and drag them over to the container on the right to assign them to placed samples.
b. Select Record Details.

6. On the Record Details screen, set the samples to NextSeq as follows.
   a. Select all of the samples in the Sample Details table. From the Sequencing Instrument drop-down list, select NextSeq.
   b. Select Apply to set this value for all selected samples.
   c. Select Next Steps.
7. On the Assign Next Steps screen, the next step is already set to Remove from Workflow. This is required by the Routing script automation.
8. Select Finish Step.
   The Routing script automation is triggered and samples are automatically routed to the first step in the NextSeq 500/550 Sequencing v1.1 protocol. This is the Library Pooling (NextSeq 500/550 v1.1) step.

NextSeq 500/550 Sequencing v1.1 Protocol

1. Run Library Pooling (NextSeq 500/550 v1.1) Step

1. In Lab View, locate the NextSeq 500/550 Sequencing v1.1 protocol. You will see your samples queued for the Library Pooling (NextSeq 500/550 v1.1) step. Select the step to proceed to the Queue screen.
2. On the Queue screen, add samples as follows.
   a. Add the samples to the Ice Bucket. In the Add Control Samples panel, add the PhiX v3 control sample to the Ice Bucket.
   b. Select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Pool Samples screen, create a pool of samples as follows.
   a. Drag samples into the Pool Creator.
   b. Name the pool. You can also accept the default name Pool #1.
   c. Select Place Samples.
5. On the Placement screen, move the pool to the container as follows.
   a. Select the pool in the Samples to be Placed area on the left. Drag it over to the container on the right.
   b. Select Record Details.
6. On the Record Details screen, select Next Steps.
   On the Assign Next Steps screen, the next step is already set to Denature and Dilute (NextSeq 500/550 v1.1).

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7. Select Finish Step.

2. Run Denature and Dilute (NextSeq 500/550 v1.1) Step

1. In Lab View, locate the NextSeq 500/550 Sequencing v1.1 protocol. You will see your pooled samples queued for the Denature and Dilute (NextSeq 500/550 v1.1) step. Select the step to proceed to the Queue screen.
2. On the Queue screen, add the pool to the Ice Bucket. Select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Placement screen, move the pool to the reagent cartridge as follows.
   a. Scan the NextSeq reagent cartridge barcode into the NextSeq Reagent Cartridge field.
   b. Place the pool of samples into the reagent cartridge.
   c. Select Record Details.
5. On the Record Details screen, create the sample sheet as follows.
   a. Under Reagent Lot Tracking, select the reagent lot used in the step. You may need to add/activate the lot on the Reagents and Controls screen.
   b. In the Step Details table, populate the fields as appropriate. The Workflow and Read 1 Cycles are required fields.
   c. In the Samplesheet Template drop-down list, the reverse complement template is selected by default. Do not change this value.
   d. In the Sample Details table, enter the Final Loading Concentration. You may select preset 225 (for PCR-free workflows) or preset 400 (for Nano workflows). You can also enter a different value.
   e. Select Generate bcl2fastq2 NextSeq SampleSheet. Clarity LIMS generates the sample sheet and attaches it and a log file to placeholders in the Files area of the Record Details screen.
   f. Download the files and validate their format and content.
   g. Select Next Steps.
6. On the Assign Next Steps screen, make sure samples are already assigned to the NextSeq 500/550 Run (NextSeq 500/550 v1.1) step.
7. Select Finish Step.

3. Run NextSeq 500/550 Run (NextSeq 500/550 v1.1) Step

When the sequencing run is finished, the integration generates a run report file. The Instrument Details section is left blank by default, unless the NextSeq 500/500 Run master step is configured to track instruments created in Clarity LIMS. For more information, refer to *Add and Configure Instruments* from the *Clarity LIMS v6 Core Functionality* documentation.

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1. In Lab View, locate the NextSeq 500/550 Sequencing v1.1 protocol. You will see your pool of samples queued for the NextSeq 500/550 Run (NextSeq 500/550 v1.1) step. Select the step to proceed to the Queue screen.
2. Add the pool to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
   On the Record Details screen, the fields in the table are read-only. Once the run completes, the integration will automatically populate the fields.
   The integration also attaches files to the Illumina Run Report, Link to Run Folder, Run Parameters, and Run Info placeholders. The Log File is attached once the next step for samples has been assigned by the Next Step - Advance automation. The integration also populates the fields in the Sample Details table. For details, refer to *NextSeq 500/550 v2.3 Configuration and User Interaction on page 1804*.
   Do not continue to step 5 and complete the step until the Illumina Run Report has been attached.
4. If the NextSeq 500/550 Run master step is configured to track instruments, select the appropriate instrument from the Instrument Used drop-down on Record Details screen, then select Save.
   For more detailed steps on adding instruments, refer to the *Add and Configure Instruments* section of the Clarity LIMS Core Functionality documentation.
5. Select Next Steps.
   On the Assign Next Steps screen, make sure the next step is already set to Mark protocol as complete.
6. Select Finish Step.

Validate Creation of Event Files

Follow the steps below to confirm that event files are created by the batch file in the destination path. The steps assume that the default configuration has been successfully imported, and support for NCS v4.0 has been configured.

This test validates that:

- Your DESTINATION_PATH is correctly configured.
- The instrument computer can access and write to the DESTINATION_PATH.
- There are no syntax errors in the Clarity LIMS batch file.

You can validate the gls_event_ncs_rta.bat batch file by executing it manually as follows.

1. Edit `gls_event_ncs_rta.bat` using any text editor (Notepad++ recommended).
   By default, this file is installed to `C:\Illumina\gls`.
2. In the code line set `DESTINATION_PATH=C:\Illumina\gls\Events\`, change `C:\Illumina\gls\Events\` to the network path of the event files directory.
   Remember to include the trailing "\" at the end of the file path.
3. Save your changes.

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Manually executing the batch file will create a dummy EndRun event file in the directory that you defined in the previous step.

Troubleshooting

If an automation trigger does not appear to run its corresponding scripts, refer to the following topics:

- *Troubleshooting Automation Worker* in the *Administration* section of the *Clarity LIMS v6 Core Functionality* documentation.
- *Troubleshooting Automation* in the *Automation* section of the *Clarity LIMS API & Database* documentation.

If an error occurs that does not provide direction on how to proceed, confirm the version of the installed Illumina NextSeq Integration Package by running the `rpm -qa | grep -i nextseq` command from the server console.

If the error is related to data parsing, retrieving run results data, or report values not appearing as expected, review the `NextSeqIntegrator.log` file. It is located at `/opt/gls/clarity/extensions/Illumina_NextSeq/v2/SequencingService`.

Additional troubleshooting information for this integration is provided on the *Illumina Instrument Integrations FAQ* on page 2598 page.

If you are unable to resolve the issue, contact the Clarity LIMS Support team. Make sure you supply the relevant information from the troubleshooting that was already performed.

NextSeq 500/550 Integration v2.3 Installation

The Illumina NextSeq 500/550 Integration v2.3 supports the integration between Clarity LIMS and the NextSeq 500/550 instrument. This document provides instructions for installing NextSeq 500/550 Integration v2.3. It also describes the components that are installed in the default configuration.

Integration Overview

Illumina NextSeq 500/550 Integration v2.3 includes the following features and functionality:

- Added support for Oracle Linux. For compatibility, refer to *NextSeq 500/550 Integration v2.3 Release Notes* on page 1841.
- A new NextSeq 500/550 v1.1 workflow that maps to lab protocols and instrument runs. This workflow includes a minor bug fix and has no additional changes from the previous version.
- Automated generation of the sample sheet. This sample sheet is used with the bcl2fastq2 v2.20.0 analysis software.
- Automated tracking of the NextSeq sequencing runs and parsing of run statistics into Clarity LIMS, which includes the following information:
  - Sequencing run metrics

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  - Sequencing run parameters
  - Real-Time Analysis v2 (RTA2) run directory location and other run-specific information
- Preconfigured Library Prep Validation v2.3.1 workflow used for validation purposes. This workflow contains a single-step protocol that models the library prep required to produce normalized libraries that are ready for the NextSeq 500/550 Sequencing v1.1 workflow. For more information, refer to *NextSeq 500/550 Integration v2.3 Validation and Troubleshooting* on page 1824.

Compatibility

The integration is compatible with the following software:

- Clarity LIMS v6.2 and later
- Secret Util v1.0 and later (v1.2 is recommended)
- IPP v2.6 and later

This integration is not fully compatible with NextSeq 500/550 Integration v1.

Supporting Documentation

- For details on installed protocols and steps, automations, generated and captured files, and rules and constraints, refer to *NextSeq 500/550 v2.3 Configuration and User Interaction* on page 1804.
- For information on validating and troubleshooting the integration, refer to *NextSeq 500/550 Integration v2.3 Validation and Troubleshooting* on page 1824.

Prerequisites

NextSeq 500/550 Integration v2.3 has the following prerequisites:

- Mount run data network-attached storage (NAS) share
- Secret Util is installed
- IPP is installed

Prerequisite 1: Mount Run Data NAS Share

Mounting the NAS share of run data is needed to capture and generate files associated with the sequencing run. To mount NAS shares that contain data from the Clarity LIMS server, use Read/Write priviledges as the glsjboss user. The following data can be mounted to the NAS share:

- Run data (eg, `\\network-storage\run_data\`)
- Clarity LIMS-created events triggered by the End Run event of the Illumina sequencing run (eg, `\\network-storage\illumina\gls_events\`)

With Read access, the Clarity LIMS server reads the following information in individual sequencing run data folders:

- Run information metadata from `<runFolderRoot>/RunInfo.xml and <runFolderRoot>/RunParameters.xml`
- Run statistics from `<runFolderRoot>/InterOp/*.bin`

The Clarity LIMS server generates the following files and information locally and stores them in Clarity LIMS:

- Sample sheet (CSV file)
- Run report (PDF file)
- Run folder root link

The Clarity LIMS server copies and stores the following files from individual sequencing run data folders in Clarity LIMS:

- `<runFolderRoot>/RunInfo.xml`
- `<runFolderRoot>/RunParameters.xml`

Prerequisite 2: Secret Util Installation

If Clarity LIMS Secret Utility is not installed or configured, use the following information to configure Secret Utility:

Installation on a hosted system with Clarity LIMS (v6.2 or later)

The Clarity LIMS installation tooling configures this installation. No additional configuration is necessary.

Installation on an on-premise Clarity LIMS instance (v6.2 or later)

Install and configure Secret Util as follows.

1. Install the ClarityLIMS-SecretUtil RPM.
2. If Secret Util is not installed or configured, you must configure it before proceeding. As the glsjboss user, run the following script:
   ```
   bash /opt/gls/clarity/config/pending/05_configure_claritylims_secretutil.sh
   ```
   For more information on installing Secret Util with the Integration Module, refer to *Install/Upgrade Secret Management for Integration Modules* in the *Clarity LIMS Core Functionality* documentation.

   For the on-premise installation, the Illumina Vault server is not available for public access.

   In the configuration, make sure the system passwords are configured correctly in File mode.
3. If a custom API username is required, configure the username and password using Secret Util:
   ```
   java -jar /opt/gls/clarity/tools/secretutil/secretutil.jar -n=INTEGRATION -u=<password> <key>
   ```

For a custom API username, set the `<key>` to `apiusers/{custom API username}` (eg, `apiusers/novaseq_user`). For more information on Secret Utility configuration, refer to the *Clarity LIMS Core Functionality* documentation.

4. Use the following command to make sure that the password is saved correctly in Secret Util:
```
java -jar /opt/gls/clarity/tools/secretutil/secretutil.jar -n=INTEGRATION
<key>
```

For more information on Secret Utility configuration, refer to the *Clarity LIMS Core Functionality* documentation.

Prerequisite 3: IPP Installation

NextSeq 500/550 Integration v2.3 depends on the configuration provided in IPP (v2.6 and later) for Clarity LIMS v6.2 and later.

If the base configuration is not installed, then install it on the Clarity LIMS server that is being used for the NextSeq integration. For details on IPP v2.6 installation and configuration, refer to the *Illumina Preset Protocols* documentation.

If you are upgrading the base configuration, make sure that the IPP package is compatible with the version of Clarity LIMS you are installing (in this case, IPP v2.6 for Clarity LIMS v6.2).

Installation

NextSeq 500/550 Integration v2.3 is distributed as the ClarityLIMS-Illumina-NextSeq-Package-v2 RPM package that must be installed on the Clarity LIMS server. This package installs the following items:

- Bash scripts used to run the service
- The `nextseq-sequencing.jar` file
- The `configure_extensions_nextseq_sequencingservice.sh` script
- Smoke test directories

When upgrading from an existing NextSeq 500/550 workflow, the following warning messages can display when the `illumina-preset-protocls-installer.sh` script is running:

- WARN Installer - Conflicting content:
- WARN Installer - Analyte UDFs: % Aligned R1
- WARN Installer - Analyte UDFs: % Aligned R2
- WARN Installer - Analyte UDFs: % Bases >=Q30 R1
- WARN Installer - Analyte UDFs: % Bases >=Q30 R2
- WARN Installer - Analyte UDFs: Cluster Density (K/mm^2) R1
- WARN Installer - Analyte UDFs: Cluster Density (K/mm^2) R2
- WARN Installer - Analyte UDFs: Clusters PF R1

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- WARN Installer - Analyte UDFs: Clusters PF R2
- WARN Installer - Analyte UDFs: Clusters Raw R1
- WARN Installer - Analyte UDFs: Clusters Raw R2
- WARN Installer - Analyte UDFs: Intensity Cycle 1 R1
- WARN Installer - Analyte UDFs: Intensity Cycle 1 R2

These messages are due to configuration changes that have increased the precision of the listed fields from 1 to 2 so that they show more decimal places. It is safe to override these warnings.

The following installation steps are required for the installation of NextSeq 500/550 Integration v2.3.

The NextSeq RPM must be installed on the Clarity LIMS server. This is where the AI node or Automation Worker is installed. For LIMS v5.x and v6.x, the automations and sequencing service use the existing Automation Worker.

Install the RPM

1. On the Clarity LIMS server, log in as the root user.
2. Run the following yum command to install the RPM:

   You must use the `--enablerepo` command line argument to enable the repo. For the repo file and the correct name to use, contact Illumina Support.

   ```
   yum install ClarityLIMS-Illumina-NextSeq-Package-v2 --enablerepo=< repo name info from support >
   ```

3. Enter `y` to confirm that you want to proceed with the RPM installation.
   After confirmation, you are prompted to install the NextSeq 500/550 workflow from the IPP RPM.

   If you have not installed the SecretUtil RPM before, this RPM installation also installs it.

   - For hosted installations, the Secret Util installation and configuration is handled by tooling.
   - For on-premise installations, if the Secret Util package is not already installed or configured, you must configure the Secret Util first. For more information, refer to *Prerequisite 2: Secret Util Installation* on page 1831.

4. Install the NextSeq 500/550 workflow as follows.
   a. As the glsjboss user, run the following command to view the complete list of IPP workflows:
      ```
      /opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o list
      ```
   b. Run the following command to install the NextSeq 500/550 Sequencing v1.1 workflow:
      ```
      /opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o install Illumina_Instruments.nextseq550-v1.1
      ```
   c. [Optional] Run the following command to install the Library Prep Validation v2.3.1 workflow that is used to validate the NextSeq 500/550 Sequencing v1.1 workflow:

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```
/opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o install
Illumina_Instruments.Library-Prep-Validation-v2.3
```

5. Run the following script to configure the service properties:
   `/opt/gls/clarity/config/configure_extensions_nextseq_sequencingservice-v2.sh`

Configure the Database Service Properties

To enable capture and generation of files associated with the sequencing run, configure the following properties using the omxProps-ConfigTool utility. The property configuration for NextSeq 500/550 v2 has the suffix .v2 and is different from the setting for v1.

Table 382 Configurable Database Properties

Property	Description	Default Value
nextseq.v2.seqservice.eventFileDirectory.1	A monitored network location used for event files.	`/mnt/gls_events`
nextseq.v2.seqservice.eventFileDirectory.99	The monitored smoke test location used for event files. This location is also used for the installation and upgrade of smoke tests.	`/opt/gls/clarity/smoketests/nextseq/monitored`
nextseq.v2.seqservice.netPathPrefixSearch.1	The network directory prefix contained in the event file.	`\\nas\network\run_data`

Property	Description	Default Value
nextseq.v2.seqservice.netPathPrefixSearch.99	The default benign smoke test search entry. This property is used for the installation and upgrade of smoke tests.	/
nextseq.v2.seqservice.netPathPrefixReplace.1	The mapped network directory mount name on the server used to access the run data directory.	/mnt/run_data
nextseq.v2.seqservice.netPathPrefixReplace.99	The default benign smoke test replace entry. This property is used for the installation and upgrade of smoke tests.	/

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Property	Description	Default Value
nextseq.v2.seqservice.eventFileDirectorySuffixes	A list of eventFileDirectory path entries used to monitor for event files. The value is one or more comma separated integers.	99 When installed, this property must be configured to point to 1 unless a validation test is being performed with the 99 version of the search, replace, and event file entries.
nextseq.v2.seqservice.netPathPrefixSearchReplaceSuffixes	A list of netPathPrefix search and replace entries for transforming Windows to Linux network paths. The value is one or more comma separated integers.	99 When installed, this property must be configured to point to 1 unless a validation test is being performed with the 99 version of the search, replace, and event file entries.

Start the Sequencing Service

1. Run the following command to start the sequencing service:

```
systemctl start nextseq_seqservice-v2
```

NextSeq Control Software v4.0 Support Configuration

In NextSeq Control Software (NCS) v4.0 the location of `NextSeq.Configuration.xml` RTA configuration file is changed. The new location is `C:\Program Files\Illumina\RTA\2.11.3\Configs`.

A new gls_events_ncs_rta.bat batch file is required to generate a valid EndRun event file. This file is available in the NextSeq 500/550 RPM at `/opt/gls/clarity/extensions/Illumina_NextSeq/v2/InstrumentIntegrations`.

Configuration

1. Download the latest batch file from the NextSeq 500/550 RPM and place it in a folder (eg, `C:\Illumina\gls`). If necessary, remove the old batch file.
2. Edit the `NextSeq.Configuration.xml` configuration file at `C:\Program Files\Illumina\RTA\2.11.3\Configs`.
3. If you cannot modify the file, then open it as an administrator. Insert the following text inside the <Processing> tag. If the batch file is not at `C:\Illumina\gls`, then change that text to the applicable path.
   ```
   <PostProcessEventFile>C:\Illumina\gls\gls_event_ncs_rta.bat</PostProcessEventFile>
   ```
4. Save the configuration file.
5. Validate the file by running RTA manually. In the command prompt, run the following command:
   ```
   "C:\Program Files\Illumina\RTA\2.11.3\RTA.exe" "." configFile="C:\Program Files\Illumina\RTA\2.11.3\Configs\NextSeq.Configuration.xml" Processing.WorkProviderTypes="OfflineBased" processedfolder="."
   ```
   The following error message displays:
   ```
   Error: No run info file found in input directory .\RunInfo.xml
   ```
6. Validate the batch file by executing it manually as follows.
   a. Using a text editor, edit the gls_events_ncs_rta.bat file.
   b. In the "`set DESTINATION_PATH=C:\Illumina\gls\Events\`" code line, change `C:\Illumina\gls\Events\` to the network path of the event files directory. Make sure to include the backslash at the end of the file path.

After manually executing the batch file, a dummy EndRun event file is created in the event files directory.

Workflows, Protocols, and Steps Installed

NextSeq 500/550 Integration v2.3 works with the NextSeq 500/550 Sequencing v1.1 workflow, which contains a single protocol with the same name. This protocol includes the following steps:

- Library Pooling (NextSeq 500/550 v1.1)
- Denature & Dilute (NextSeq 500/550 v1.1)
- NextSeq 500/550 Run (NextSeq 500/550 v1.1)

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For descriptions of the protocol and the steps, refer to *NextSeq 500/550 v2.3 Configuration and User Interaction* on page 1804. For instructions on using the Library Prep Validation v2.3.1 workflow to validate the automated sample sheet generation, refer to *NextSeq 500/550 Integration v2.3 Validation and Troubleshooting* on page 1824.

Instrument Software

The NCS is divided into the following modules:

- NCS—Controls the instrument operation, including various configuration settings. This software is installed and runs on the instrument.
- Real-Time Analysis 2 (RTA2)—Performs image processing and base calling (primary analysis). The software makes sure that data files are created and copied to the final destination folder and is installed and runs on the instrument.

For more information on NCS, refer to the NextSeq 500/550 documentation at support.illumina.com.

Instrument Integration

The instrument integration must be performed and maintained by Illumina Support. Illumina Support requires remote access to the instrument while it is idle.

Illumina Support has created batch files that use custom scripts during the key events of a sequencing run. When these batch files are used, they read the event information and write it in a TXT event file at the same network share location that the instrument uses to write the run data. Another process running on the Clarity LIMS server receives the event files and takes the appropriate actions.

The sequencing service monitors the end of the run event. This event is used to capture key step data and files and parse run statistics for output custom fields.

For NCS v4.0 and later, refer to the configuration steps in *NextSeq Control Software v4.0 Support Configuration* on page 1836. For NCS versions before v4.0, perform the following steps.

Configure Batch Files

When the instrument is running, the final destination for the run data is a network storage path. The software is configured with a network storage path root (eg, `\\network-storage\illumina\`). Each sequencing run generates a unique run ID, which is appended to create a unique data run directory (eg, `\\network-storage\illumina\110419_InstrumentName_0001_ARG1234567`).

The Clarity LIMS batch files must be configured to write to a directory within the network storage path root. This directory is typically named `gls_events`, but the directory name can be different as long as no spaces are used.

Before configuring the batch files, do the following:

1. Back up the `NextSeq.Configuration.xml` configuration file to `C:\Illumina\RTA\Configs\`.
2. Make sure that the instrument is idle.
3. Shut down NCS.

4. Set up the directory structure as follows.
   a. Create a directory (`C:\Illumina\gls` is recommended) on the local computer to hold the batch file.

      Because of Windows 10 software restriction policies, this folder must be under C:\Illumina. Otherwise, the script is prohibited from running.

   b. Create a directory (eg, `gls_events`) on the NAS to hold the event files.

Configure the batch files as follows.

1. Determine the site or instrument specific network storage path root.
2. Change the DESTINATION_PATH line to the name of the event file directory in the `gls_event_ncs_rta.bat` batch file:

   Make sure to include the trailing \ in the DESTINATION_PATH line. Refer to the following example:

   ```
   set DESTINATION_PATH=\\network-storage\illumina\gls_events\
   ```

3. Copy the DESTINATION_PATH and paste it into the Windows Explorer address bar.
4. Make sure that the network location is accessible and opens from the instrument.

The batch file needs to be deployed to the NextSeq 500/550 instrument. This file must be deployed to its own directory (`C:\Illumina\gls` is recommended) on the instrument computer so that it is not overwritten or removed during an instrument software update.

Deploy the batch file as follows.

1. Download the batch file.
2. Copy the file to the directory that you created on the instrument computer (eg, `C:\Illumina\gls`).
3. From the Command Prompt, run the following command to list the contents of the directory:
   ```
   dir C:\Illumina\gls\
   ```
4. Make sure that the name of the batch file does not contain any special or hidden characters.

Connect Batch Files to Instrument Events

Update the NCS configuration files as follows.

1. Restart the computer and make sure that the instrument is off.
2. Using Task Manager, make sure that NCS is not auto-launched by Windows.
3. If NCS is being auto-launched, remove it from the auto-launch list and restart the computer.
4. Make a backup copy of the RTA configuration XML file and name it `NextSeq.Configuration.xml`.
5. Edit the file.
6. Save the edited file.
7. Restart the computer.
8. Turn the instrument on and launch NCS.

9. Open the configuration file and make sure that the changes were saved.

Connect to RTA End Run Event

1. Edit the NextSeq.Configuration.xml configuration file at `C:\Illumina\RTA\Configs\`.
2. Insert the following text inside the <Processing> tags:

```
<PostProcessEventFile>C:\Illumina\gls\gls_event_ncs_
rta.bat</PostProcessEventFile>
```

3. Validate the RTA configuration changes as follows.
   c. From the command prompt, run the following command:

```
C:\Illumina\RTA\RTA.exe "."
configFile="C:\Illumina\RTA\Configs\NextSeq.Configuration.xml"
Processing.WorkProviderTypes="OfflineBased" processedfolder="."
```

   d. If the validation is successful, the following warning message displays. Select OK.

```
Error: No run info file found in input directory .\RunInfo.xml
```

   If the configuration file has an error, the command returns specifics about the problem. Refer to the following example:

```
Error: While loading Configuration
"c:\illumina\rta\configs\NextSeq.Configuration.xml"
There is an error in XML document <83, 5>
Error loading xml file: Unknown node 'GLS' at line 83 pos 5
```

4. Restart the computer.
5. Turn on the instrument and start NCS.
6. Open the `NextSeq.Configuration.xml` configuration file and make sure that the changes were saved.

Validation

Sample Sheet Generation

For instructions on how to validate the automated sample sheet generation, refer to *NextSeq 500/550 Integration v2.3 Validation and Troubleshooting* on page 1824.

Manual Invocation of Event Files

The manual invocation of an event file produces an output that contains the following information:

- Filename (eg, `event-EndRun-07295667.txt`)
- cycleNumber (eg, 318)
- runFolder (eg, `"D:\Illumina\NextSeqTemp\230815_M99999_0028_FC1234567-ABCDE"`)
- netFolder (eg, `\\network-folder\Run_Data\230815_M99999_0028_FC1234567-ABCDE`)
- readType (eg, 4)
- eventType (eg, EndRun)

- softwareType (eg, NCS)
- finishDate (eg, 2023-08-15)

If an event file is triggered automatically during a NextSeq 500/550 run, then the completion event does not appear until the run has completed and RTA completes primary analysis.

For instructions on validating the creation of event files, refer to *NextSeq 500/550 Integration v2.3 Validation and Troubleshooting* on page 1824.

Instrument Sequencing Run

The instrument sequencing run test validates that the Clarity LIMS batch file is connected properly and invoked on the instrument events. Before validating the batch file, make sure that you have the following prerequisites are met:

- You have access to the NAS share.
- The default configuration has been successfully imported.
- Manual invocation of the event files has been validated. This validation checks for the following information:
  - The DESTINATION_PATH is configured correctly.
  - The instrument computer can access and write to the DESTINATION_PATH.
  - There are no syntax errors in the Clarity LIMS batch script.

For more information on event file validation, refer to *NextSeq 500/550 Integration v2.3 Validation and Troubleshooting* on page 1824.

The sequencing service processes and archives event files, which can cause validation issues while the service is running. You can make the following changes to avoid losing the event files that are you attempting to validate:

- Modify the FINAL_EXTENSION value in the Clarity LIMS batch file so that the file extension is .test instead of .txt. The service only processes and archives TXT files. Make sure that you change FINAL_EXTENSION back to .txt after manual validation.
- Monitor the `NextSeqIntegrator.log` file, which logs the file name and contents of each event file that is processed. The log file is at `/opt/gls/clarity/extensions/Illumina_NextSeq/v2/SequencingService`.

Validate the sequencing run as follows.

1. During the run, monitor the contents of the gls_events directory.
2. After the run is complete and the RTA completes primary analysis, make sure that a final EndRun event displays (eg, `event-EndRun-11043279.txt`).

NextSeq 500/550 Integration v2.3 Release Notes

These Release Notes describe the key changes to software components for the Clarity LIMS NextSeq 500/550 Integration Package v2.3.

Compatibility

This version of the integration package is compatible with the following software:

- Clarity LIMS v6.2 and later
- NGS Extensions Package v5.23 and later
- Illumina Preset Protocols (IPP) v2.6.0 and later
- NextSeq Control Software (NCS) v4.0 (Standalone mode only)
- Oracle Linux (64-bit) v8.8

New Features

- Updates Java and third-party dependency libraries.
- Updates Groovy to v3.0.7.

Defect Repairs

- Fixed SQL Injection (CVE-2022-31197) and Information Disclosure (CVE-2020-15522) vulnerabilities.

Known Issues

- The Run Type custom field is not updated under Step Details in the NextSeq 500/550 Run step.
- Missing contents in the Instrument Details section of the run report.

NextSeq 500/550 Configuration and User Interaction

The Illumina NextSeq 500/550 Integration Package v2.2 supports the integration of Clarity LIMS to Illumina NextSeq 500 and 550 sequencing systems.

The integration allows for automated tracking of an Illumina sequencing run in Clarity LIMS, which includes tracking instrument run status, generating run report, and capturing and parsing run statistics. In addition, this integration provides automated generation of a sample sheet file for use with bcl2fastq2 v2.20.0 analysis software.

This document describes the integration between Clarity LIMS and the NextSeq system. It includes information about protocols and automations, configuration options, installed components, and rules and constraints.

See *NextSeq 500/550 v2.2 Validation and Troubleshooting Guide* for instructions on validating and troubleshooting the NextSeq 500/550 Integration Package.

Integration Overview

The NextSeq 500/550 Integration Package v2.2 includes the following items:

- Automated generation of a sample sheet for use with bcl2fastq2 v2.20.0 analysis software.
- Automated tracking of the NextSeq sequencing run and parsing of run statistics into Clarity LIMS, including:

- Run status and metrics of sequencing run
- Sequencing run parameters
- Real-Time Analysis v2 (RTA2) run directory location and other run-specific information

Prerequisites and Assumptions

It is assumed that samples enter the NextSeq 500/550 Sequencing v1.0 workflow as normalized libraries and have reagent labels attached.

That is, before they are assigned to the workflow:

- Samples have been accessioned into Clarity LIMS.
- Samples have been run through QC and library prep.
- Samples have been normalized, and the value is captured in a field called Normalized Molarity (nM).

For more information on sample accessioning, refer to *Sample Accessioning* and *Upload and Modify Samples* in the Getting Started section of the Clarity LIMS Core Functionality documentation.

You can assign samples to workflows automatically, using a routing script, or manually—from the Projects & Samples dashboard. Refer to *Assign and Process Samples* in the Clarity LIMS Core Functionality documentation.

Workflows, Protocols, and Steps

NextSeq Integration Package v2.2 includes the NextSeq 500/550 Sequencing v1.0 workflow, which contains a single protocol of the same name.

The NextSeq 500/550 Sequencing v1.0 protocol includes the following steps:

- Library Pooling (NextSeq 500/550 v1.0)
- Denature & Dilute (NextSeq 500/550 v1.0)
- NextSeq 500/550 Run (NextSeq 500/550 v1.0)

Step Automations

The following table lists the automations configured on the NextSeq 500/550 Sequencing v1.0 steps. Automation details are provided in the configuration details sections following the table. See *NextSeq 500/550 v2.2 Validation and Troubleshooting Guide* for details on the validation workflow.

Table 383 NextSeq 500/550 Sequencing System v1.0 Protocol Step Automation

Step	Automation
1. Library Pooling (NextSeq 500/550 v1.0)	• Set Next Step - Advance

Step	Automation
2. Denature and Dilute (NextSeq 500/550 v1.0)	• Generate bcl2fastq2 NextSeq 500/550 SampleSheet • Set Next Step - Advance
3. NextSeq 500/550 Run (NextSeq 500/550 v1.0)	• Set Next Step - Advance

Terminology

The NextSeq 500/550 Integration Package v2.2 supports Clarity LIMS v5.1 and later.

As of Clarity LIMS v5, the term user-defined field (UDF) has been replaced with custom field in the user interface. However, the API resource is still called UDF. There are two types of custom fields:

- Master step fields—Configured on master steps. Master step fields only apply to the following: The master step on which the fields are configured. The steps derived from those master steps.
- Global fields—Configured on entities (eg, submitted sample, derived sample, measurement, etc.). Global fields apply to the entire Clarity LIMS system.

Step 1: Library Pooling (NextSeq 500/550 v1.0)

The Library Pooling (NextSeq 500/550 v1.0) step is derived from the Library Pooling v1.0 master step. Libraries are placed into pools manually.

Set Next Step - Advance Automation

This automation is automatically triggered on exit of the Record Details screen. The automation advances samples to the next step in the protocol.

The default command line is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-t false \
-h false \
-exp 'nextStep = ::ADVANCE::' \
-log {compoundOutputFileLuid0}"
```

Master Step Fields

This field is configured on the Library Pooling (NextSeq 500/550 v1.0) step and displays on the Record Details screen at run time.

The following table shows field configuration details.

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Table 384 Library Pooling (NextSeq 500/550 v1.0) Master Step Field Configuration

Field Name	Field Type	Field Constraints/Options
Comment	Multiline Text	• Required: No • Read Only: No

Global Fields

The following table lists the global custom fields configured to display on the Queue and Ice Bucket screens of the Library Pooling (NextSeq 500/550 v1.0) step. Most of these fields show in the expanded view only.

Table 385 Global Custom Field Configuration for Library Pooling (NextSeq 500/550 v1.0)

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Configured on submitted sample:			

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Application	Text Dropdown	• Required: No • Read Only: No • Custom Entries: Yes	• TruSeq mRNA sequencing • TruSeq DNA sequencing (large genome de novo) • TruSeq DNA sequencing (large genome reseq) • TruSeq DNA sequencing (small genome de novo) • TruSeq DNA sequencing (small genome reseq) • Nextera DNA sequencing • TruSeq Custom Amplicon sequencing • ChIP-sequencing • Exome sequencing • Mate pair sequencing • Small RNA sequencing
Pooling	Text Dropdown	• Required: No • Read Only: No • Custom Entries: Yes	• Yes • No
Read Length	Text	• Required: No • Read Only: No	Not applicable
Sequencing Coverage	Text	• Required: No • Read Only: No	Not applicable

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Sequencing Method	Text Dropdown	• Required: No • Read Only: No • Custom Entries: Yes	• Single Read • Paired End Read • Indexed Single read • Indexed Paired End Read
Configured on derived sample:			
Normalized Molarity (nM)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2

User Interaction

1. On the Queue screen, add the samples to the Ice Bucket.
2. In the Add Control Samples panel, add the PhiX v3 control sample to the Ice Bucket.
3. Select View Ice Bucket.
4. On the Ice Bucket screen, select Begin Work.
5. On the Pool Samples screen, create a pool of samples by dragging samples into the Pool Creator. Name the pool or accept the default name (Pool #1).
6. Select Place Samples.
7. On the Placement screen, select the pool in the Samples to be Placed area on the left and drag it to the container on the right.
8. Select Record Details.
9. On the Record Details screen, select Next Steps.
10. On the Assign Next Steps screen, make sure the next step is set to Denature and Dilute (NextSeq 500/500 v1.0).
11. Select Finish Step.

Step 2: Denature and Dilute (NextSeq 500/550 v1.0)

In this step, pooled libraries are denatured and diluted and placed into the reagent cartridge that is loaded into the NextSeq instrument.

Generate bcl2fastq2 NextSeq SampleSheet Automation

This automation is triggered by a button on the Record Details screen.

This automation generates the sample sheet and attaches it to the step. For details, see the following section on sample sheet generation.

The default command line is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-t false \
-h false \
-exp 'nextStep = ::ADVANCE::' \
-log {compoundOutputFileLuid3}"
```

Set Next Step - Advance Automation

This automation is automatically triggered on exit of the Record Details screen.

This automation advances samples to the next step in the protocol. The automation also invokes the setUDF script, which sets the value of the Progress field. Clarity LIMS updates this field value as samples progress through the workflow steps. The Progress field does not display in the Clarity LIMS user interface. However, it does display in the LabLink Collaborations Interface so that collaborators can see the status of their samples.

The default command line is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-t false \
-h false \
-exp 'nextStep = ::ADVANCE::' \
-log {compoundOutputFileLuid3}"
```

Master Step Fields

There are 10 fields configured on the Denature and Dilute (NextSeq 500/550 v1.0) step. At run time, these fields display on the Record Details screen, in the Step Data table. The fields are manually populated. Their values are used to generate the sample sheet.

The following table lists field configuration details.

Table 386 Denature and Dilute (NextSeq 500/550 v1.0) Master Step Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Adapter	Text	• Required: No • Read Only: No	Not applicable
Adapter Read 2	Text	• Required: No • Read Only: No	Not applicable
Experiment Name	Text	• Required: No • Read Only: No	Not applicable
Mask Adapter	Text	• Required: No • Read Only: No	Not applicable
Mask Adapter Read 2	Text	• Required: No • Read Only: No	Not applicable
Read 1 Cycles	Numeric Dropdown	• Required: No • Read Only: No • Custom Entries: Yes	• 251 (Default) • 151 • 101 • 76 • 51 • Range From: 0 to 1000 • Decimal Places Displayed: 0
Read 2 Cycles	Numeric Dropdown	• Required: No • Read Only: No • Custom Entries: Yes	• 251 • 151 • 101 • 76 • 51 • Range From: 0 to 1000 • Decimal Places Displayed: 0

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
SampleSheet Template	Text Dropdown	• Required: No • Read Only: No • Custom Entries: No	• BCL2FASTQ_ Reverse_ Complement_ Samplesheet.csv (default) • BCL2FASTQ_ Samplesheet.csv
Workflow	Text Dropdown	• Required: No • Read Only: No • Custom Entries: No	• Assembly • Custom Amplicon • Enrichment • GenerateFASTQ • LibraryQC • Metagenomics • PCR Amplicon • Resequencing

Groups of Defaults

The following group of defaults are configured for Denature and Dilute (NextSeq 500/550 v1.0) master step custom fields:

- Resequencing
  - Read 1 Cycles: 251
  - Workflow: Resequencing
- Custom Amplicon
  - Read 1 Cycles: 251
  - Workflow: Custom Amplicon
- PCR Amplicon
  - Read 1 Cycles: 251
  - Workflow: PCR Amplicon
- Enrichment
  - Read 1 Cycles: 251
  - Workflow: Enrichment
- Library QC

  - Read 1 Cycles: 251
  - Workflow: LibraryQC
- Metagenomics
  - Read 1 Cycles: 251
  - Workflow: Metagenomics
- GenerateFastQ
  - Read 1 Cycles: 251
  - Workflow: GenerateFastQ
- Assembly
  - Read 1 Cycles: 251
  - Workflow: Assembly

Global Fields

The following table lists the global fields that are configured to display on the Queue, Ice Bucket, and Record Details screens of the Denature and Dilute (NextSeq 500/550 v1.0) step.

Table 387 Global Custom Field Configuration for Denature and Dilute (NextSeq 500/550 v1.0) Step

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Configured on submitted sample:			
Progress	Text	• Required: No • Read Only: No • Custom Entries: Yes	Not applicable
Read Length Displays on Queue & Ice Bucket screens	Text	• Required: No • Read Only: No	Not applicable
Sequencing Method Displays on Queue & Ice Bucket screens	Text Dropdown	• Required: No • Read Only: No • Custom Entries: Yes	• Single Read • Paired End Read • Indexed Single Read • Indexed Paired End Read

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Configured on derived sample:			
Final Loading Concentration Displays on Record Details screen	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 225 • 400

Step File Placeholders

Placeholders for the following files are configured on the Record Details screen of the Denature and Dilute (NextSeq 500/550 v1.0) step:

- Lab Tracking Form (manually generated)
- bcl2fastq2 Samplesheet (autogenerated)
- bcl2fastq2 Samplesheet Generation Log (autogenerated)
- Log File (autogenerated)

Table 388 Sample Sheet Files & Placeholders Generated by Denature & Dilute (NextSeq 500/550 v1.0) Step

Item	Description
Lab Tracking Form placeholder	This placeholder in Clarity LIMS allows for manually attaching a lab-specific tracking form to the step.
bcl2fastq2 SampleSheet	This CSV file is automatically generated by Clarity LIMS for use with the bcl2fastq2 v2.20.0 analysis software. It can be opened as a text file or as an MS Excel spreadsheet.
bcl2fastq2 SampleSheet Generation Log	Automatically generated by Clarity LIMS, this log file captures any errors that Clarity LIMS can encounter when generating the sample sheet.
Log File	Automatically generated by Clarity LIMS, this log file captures the status of the EvaluateDynamicExpression script that is launched by the Set Next Step Advance automation.

User Interaction

1. On the Queue screen, add the pool to the Ice Bucket and select View Ice Bucket.
2. On the Ice Bucket screen, select Begin Work.
3. On the Placement screen, scan the barcode of the NextSeq reagent cartridge into the NextSeq Reagent Cartridge field. Place the pool of samples into the reagent cartridge.
4. Select Record Details.
5. On the Record Details screen under Reagent Lot Tracking, select the reagent lot used in the step. If necessary, add/activate the lot on the Reagents and Controls screen.
6. Populate the fields in the Step Details table.
   Workflow and Read 1 Cycles are required fields.
7. In the Sample Details table, enter a Final Loading Concentration value. Select from the two preset options: 225 (for PCR-free workflows) or 400 (for Nano workflows). You can also enter a different value manually.
8. Select Generate bcl2fastq2 NextSeq SampleSheet.
9. Clarity LIMS generates the samplesheet and attaches it and a log file to placeholders in the Files area of the Record Details screen. Download the files and validate their format and content.
10. Select Next Steps.
    On the Assign Next Steps screen, samples are assigned to the NextSeq 500/550 Run (NextSeq 500/550 v1.0) step.
11. Select Finish Step.

Step 3: NextSeq 500/550 Run (NextSeq 500/550 v1.0)

In this step, pooled samples are sequenced on the NextSeq 500/550 instrument and the run metrics are recorded in Clarity LIMS.

Set Next Step - Advance Automation

This automation is automatically triggered on exit of the Record Details screen. The automation advances samples to the next step in the protocol.

The default command line is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-t false \
-h false \
-exp 'nextStep = ::ADVANCE::' \
-log {compoundOutputFileLuid5}"
```


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Master Step Fields

There are 16 fields configured on the NextSeq 500/550 Run (NextSeq 500/550 v1.0) step. These fields display on the Record Details screen at run time. Some of the field values must be completed manually, and the remaining fields are automatically populated at the end of the run.

The following table lists field configuration details.

Table 389 NextSeq 500/550 Run (NextSeq 500/550 v1.0) Master Step Field Configuration

Field Name	Field Type	Filed Constraints/Options	Preset Value/Additional Options and Drop-down Items
Chemistry	Text	• Required: No • Read Only: Yes	Not applicable
Comment	Multiline Text	• Required: No • Read Only: No	Not applicable
Experiment Name	Text	• Required: No • Read Only: Yes	Not applicable
Finish Date	Date	• Required: No • Read Only: Yes	Not applicable
Flow Cell ID	Text	• Required: No • Read Only: Yes	Not applicable
Index 1 Read Cycles	Numeric	• Required: No • Read Only: Yes	Decimal Places Displayed: 0
Index 2 Read Cycles	Numeric	• Required: No • Read Only: Yes	Decimal Places Displayed: 0
Output Folder	Text	• Required: No • Read Only: Yes	Not applicable
PR2 Bottle ID	Text	• Required: No • Read Only: Yes	Not applicable
Read 1 Cycles	Numeric	• Required: No • Read Only: Yes	Decimal Places Displayed: 0
Read 2 Cycles	Numeric	• Required: No • Read Only: Yes	Decimal Places Displayed: 0
Reagent Cartridge ID	Text	• Required: No • Read Only: Yes	Not applicable

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Field Name	Field Type	Filed Constraints/Options	Preset Value/Additional Options and Drop-down Items
Run ID	Text	• Required: No • Read Only: Yes	Not applicable
Run Type	Text	• Required: No • Read Only: Yes	Not applicable
Status	Text	• Required: No • Read Only: Yes	Not applicable
Workflow	Text	• Required: No • Read Only: Yes	Not applicable

Global Fields

There are several sample and measurement global fields configured to display on the Record Details screen of the NextSeq 500/550 Run (NextSeq 500/550 v1.0) step. These fields are populated at the end of the sequencing run.

Step File Placeholders

Placeholders for the following files are configured on the Record Details screen of the NextSeq 500/550 Run (NextSeq 500/550 v1.0) step.

- Illumina Run Report (autogenerated)
- Link to Run Folder (autogenerated)
- Run Parameters (autogenerated)
- Run Info (autogenerated)
- Lab Tracking Form (manually generated)
- Log File (autogenerated)

User Interaction

1. On the Queue screen, add the pool to the Ice Bucket and select View Ice Bucket.
2. On the Ice Bucket screen, select Begin Work.
   On the Record Details screen, the fields in the table are read-only. After the run completes, the integration automatically:
   - Populates the fields.
   - Attaches files to the Illumina Run Report, Link to Run Folder, Run Parameters, and Run Info placeholders.

- Populates the fields in the Sample Details table.

3. Select Next Steps.
4. On the Assign Next Steps screen, make sure that the next step is set to Mark protocol as complete.
5. Select Finish Step.

Sample Sheet Generation

Sample sheet generation occurs on the step before the sequencing run Denature and Dilute (NextSeq 500/550 v1.0) step. Samples are placed on the container to be loaded in the instrument. The default configuration provides the Generate bcl2fastq2 NextSeq SampleSheet automation.

The Generate bcl2fastq2 NextSeq SampleSheet automation uses the Template File Generator (DriverFileGenerator.jar) and a template file to generate a CSV format file for use with bcl2fastq2 v2.20.0 analysis software. The sample sheet content is determined by the fields that display on the Record Details screen of the step (in the Step Data table) and the values entered into the fields. Templates can be customized to create the sample sheet. If additional columns are required by the lab, then they can be inserted.

Sequencing Results Parsing

The NextSeq 500/550 Run (NextSeq 500/550 v1.0) step records information for the flow cell lanes and generates a report summarizing the results. In addition, run parameters, run info, and a link to the run folder are automatically captured.

Generated and Captured Files

The following table lists the run information files, reports, placeholders, and links that Clarity LIMS automatically generates or capture during a sequencing run.

Table 390 Run Information Generated or Captured by NextSeq 500/550 Run (NextSeq 500/550 v1.0) Step

Item	Description
Run Info Run Parameters	These XML files are captured automatically by Clarity LIMS from the instrument run folder. They include the key run parameters, many of which are parsed out into key step global custom fields.
Link to Run Folder	Automatically generated by Clarity LIMS, and is a link to the network run folder where the data that was captured from the instrument during the run is stored.

Item	Description
Illumina Run Report	Automatically generated by Clarity LIMS, this report provides key information about the run and the samples on the flow cell. Information includes the flow cell ID, run directory location, and primary analysis metrics for the instrument, summarized per flow cell lane for the entire run, and individual reads if there are paired-end runs. These metrics are compared against the instrument per lane averages, calculated using metrics from the last 5 sequencing runs. Any values outside of 1 standard deviation are highlighted.
Lab Tracking Form	This placeholder in Clarity LIMS allows you to attach a lab-specific tracking form to the step manually.

Metadata

The following list includes the metadata that Clarity LIMS automatically captures from the Illumina sequencing software as part of a sequencing run. This information is gathered from various run result files and events.

- Chemistry
- Experiment Name – entered in software
- Finish Date*—Run completion date
- Flow Cell ID
- Index 1 Read Cycles—Intended Index cycles
- Index 2 Read Cycles—Intended Index cycles
- Output Folder—Run folder root
- PR2 Bottle ID
- Reagent Cartridge ID
- Read 1 Cycles
- Read 2 Cycles
- Run ID—The unique run ID
- Run Type

- Status—Current status of the sequencing run on the instrument
- Workflow

If the End Run event contains a date in the format YYYY-MM-DD, Finish Date is set to the date in the event file. If the End Run event does not contain a date or the date is in the wrong format, Finish Date is set to the date when the event file is processed.

Primary Analysis Metrics

The following table lists the Real-Time Analysis v2 (RTA2) primary analysis metrics that Clarity LIMS automatically captures and records, per read, for samples in each flow cell lane. These metrics are captured after run completion and are stored as global custom fields in the Record Details screen Sample Details table. Per read and per lane metrics are viewable by expanding the output.

Table 391 RTA Primary Analysis Metrics Captured by NextSeq 500/550 Run (NextSeq 500/550 v1.0) Step

Per Read Clarity LIMS Field Name (stored on derived sample/analyte input to the step)	Per Lane Clarity LIMS Field Name (stored in measurement placeholders in Record Details screens Sample Details table)
% Aligned R1	% Aligned R1
% Aligned R2	% Aligned R2
% Bases >=Q30 R1	% Bases >=Q30 R1
% Bases >=Q30 R2	% Bases >=Q30 R2
% Error Rate R1	% Error Rate R1
% Error Rate R2	% Error Rate R2
	% Phasing R1
	% Phasing R2
	% Prephasing R1
	% Prephasing R2
	%PF R1
	%PF R2
	Cluster Density (K/mm^2) R1
	Cluster Density (K/mm^2) R2
Intensity Cycle 1 R1	Intensity Cycle 1 R1

Per Read Clarity LIMS Field Name (stored on derived sample/analyte input to the step)	Per Lane Clarity LIMS Field Name (stored in measurement placeholders in Record Details screens Sample Details table)
Intensity Cycle 1 R2	Intensity Cycle 1 R2
	Reads PF (M) R1
	Reads PF (M) R2
Yield PF (Gb) R1	Yield PF (Gb) R1
Yield PF (Gb) R2	Yield PF (Gb) R2

The sequencing service runs on the Clarity LIMS server. The service detects event files that the instrument software (RTA2) produces as the run progresses, which tells the service where to find the run data. As the run data is written out and the End Run event is detected, the data is matched to the step. This matching is based on the reagent cartridge ID that was entered/scanned in the Denature and Dilute (NextSeq 500/550 v1.0) step. Read-only field values on the Record Details screen are populated accordingly. When finished processing the end run event and updating the fields in Clarity LIMS, the sequencing service generates the report and attaches it to the step.

User Interaction

1. Begin the step process and wait until the sequencing service has finished processing the run and the run report is generated and attached.
2. On the Record Details screen, the read-only field values are automatically populated as the instrument runs.
   After the run has completed:
   - The read-only fields are populated.
   - The Illumina Run Report is generated and attached.
   - The Link to Run Folder is generated and attached.
   - The Run Parameters and Run Info files are attached.
3. [Optional] In the Files section, upload the Lab Tracking Form.
   The steps are complete. The Log File is attached after the next step for samples is assigned by the Next Step, Advance automation.

   Do not complete the step until the Illumina Run Report is attached to the step in Clarity LIMS.

Components Installed

This integration requires installation of the Illumina Preset Protocols (IPP). For details, see Release Notes.

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Scripts and Files Installed

The following table lists the scripts and files installed in the Illumina NextSeq 500/550 Integration Package v2.2 RPM.

Table 392 Illumina NextSeq 500/550 Integration Package v2.2 Scripts and Files Installed

Files Installed	Location and Description
configure_extensions_nextseq_sequencingservice-v2.sh	/opt/gls/clarity/config/ Script that installs the service properties in the database.
log4j2.xml	`/opt/gls/clarity/extensions/Illumina_NextSeq/v2/SequencingService/conf` File containing the settings for the sequencing jar logging.
nextseq-sequencing-v2.jar	`/opt/gls/clarity/extensions/Illumina_NextSeq/v2/SequencingService` Jar file containing API-based Clarity LIMS extensions used for capturing run results and report generation.
InterOp libraries	`/opt/gls/clarity/extensions/Illumina_NextSeq/v2/lib` Illumina shared library for parsing InterOp data files.
• BCL2FASTQ_Reverse_Complement_Samplesheet.csv • BCL2FASTQ_Samplesheet.csv (Installed from the IPP)	`/opt/gls/clarity/extensions/conf/driverfiletemplates` Template file used for file generation. In this integration, the reverse complement template is used by default.

Database Properties Installed

The following table lists the database properties installed with Illumina NextSeq 500/550 Integration Package v2.2.

Additional properties, each with the 99 suffix appended to their name, are also installed and intended for use by the Clarity LIMS Support team in automated validation tests. Those properties are not listed in the table.

Sequencing runs are matched using the reagent cartridge ID and the sequencing steps base name. The base name is NextSeq 500/550 Run (NextSeq 500/550 v1.0). Do not change the base name. The name is expected by the sequencing service that captures instrument run results. The base name is stored in the sequenceProcessBaseName database property. If this name is changed without the database property being updated, the 'reagent cartridge ID <-> sequencing step base name' matching system fails. If necessary, modify the step name by editing or adding text after the base name portion, as it is not used in the matching system. For example, change NextSeq 500/550 Run (NextSeq 500/550 v1.0) to NextSeq 500/550 Run (NextSeq 500/550 v1.0) v2.2.

Table 393 Database Properties Installed with the Illumina NextSeq 500/550 Integration Package

Property	Description	Default Value
nextseq.v2.seqservice.sequenceProcessBaseName (Installed from the IPP)	Sequencing process type / master step base display name. Partial matching is used to look up the process type / master step.	NextSeq 500/550 Run (NextSeq 500/550 v1.0)
nextseq.v2.seqservice.eventFileDirectory.1	A network location monitored for event files (eg, /mnt/illumina/gls_events/')	/mnt/gls_events
nextseq.v2.seqservice.netPathPrefixSearch.1*	The network directory prefix contained in the event file - most likely in Windows format.	\\nas\network\run_data

Property	Description	Default Value
nextseq.v2.seqservice.netPathPrefixReplace.1	The mapped network directory mount name on the server used to access the run data directory (eg, /mnt/network/data')	/mnt/run_data
nextseq.v2.seqservice.eventFileDirectorySuffixes	A list of eventFileDirectory path entries to monitor for event files. The value is one or more comma-separated integers.	99 Configured on install to point to 1.
nextseq.v2.seqservice.netPathPrefixSearchReplaceSuffixes	A list of netPathPrefix search and replace entries for transforming Windows to Linux network paths. The value is one or more comma-separated integers.	99 Configured on install to point to 1.

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Property	Description	Default Value
nextseq.v2.seqservice.runReportViewsVersion	The current version of the Run Report views in the database. The value 0 represents the state before the views are created. This property is automatically updated by the run report.	0
nextseq.v2.seqservice.ignoreUnmatchedContainerIds	A flag indicating if event files that cannot be matched to reagent cartridges in Clarity LIMS should be archived after a certain time (true), or continually reprocessed (false).	false Note: To prevent the gls_events file directory from becoming cluttered, it is recommended that the value of this property is set to true.
nextseq.v2.seqservice.ignoreUnmatchedContainerIds WaitDays	The number of days between when the event is created and the event file is archived.	14
nextseq.v2.seqservice.synchronizationPeriod	Invocation period in seconds.	60

*It is possible to configure support for multiple, identical seqservice.netPathPrefixSearch property values.

Reagent Categories/Label Groups Installed

Reagent categories/label groups are installed with the IPP workflow configuration slices.

The NextSeq Reagent Kit is included in the Illumina NextSeq Integration.

Control Types Installed

The PhiX v3 control type is included in the Illumina NextSeq Integration.

Container Types Installed

The NextSeq Reagent Cartridge container type is included in the Illumina NextSeq Integration.

All one-dimensional container types with both numeric rows and numeric columns are supported.

Instrument Integration

To make sure that the Illumina instrument warranty remains valid, the instrument integration must be performed and maintained by the Clarity LIMS Support team. To perform this integration, the Support team requires remote access to the instrument while it is idle.

To configure the Illumina instrument for use with the Illumina NextSeq Integration, the Support team:

1. Creates a directory on the local computer to hold the batch files. These batch files write event files to the network attached storage (NAS) shares.
2. Creates a directory on the NAS to hold the event files.
3. Modifies Illumina software configuration files to call the batch files that create the event files.
4. Updates sequencing service default database properties to match the specifics of the installation.

Rules and Constraints

This integration operates with the following constraints:

- The reagent cartridge ID must be unique. Avoid multiple reagent cartridge containers in the system with identical names.
- The reagent cartridge ID must be scanned as the reagent cartridge Container Name on the Denature and Dilute (NextSeq 500/550 v1.0) step.

NextSeq 500/550 Integration v2.2 Validation and Troubleshooting

This guide explains how to validate the installation of the Illumina NextSeq 500/550 Integration Package v2.2.

The validation process involves:

- Running samples through the Library Prep Validation v2.0.4 workflow. This workflow is installed by Illumina Preset Protocols (IPP) v2.3 and contains a single-step protocol that models the library prep required to produce normalized libraries. At the end of the step, the normalized libraries are automatically advanced to the NextSeq 500/550 Sequencing v1.0 workflow.
- Running normalized libraries through the NextSeq 500/550 Sequencing v1.0 workflow. This validates the automated generation of a sample sheet for use with bcl2fastq2 v2.20.0 analysis software. It also validates the automated tracking of the NextSeq sequencing run and parsing of run statistics into Clarity LIMS, including:
    - Run status and metrics of sequencing run
    - Sequencing run parameters
    - Real Time Analysis v2 (RTA2) run directory location and other run specific information

As of Clarity LIMS v5, the term user-defined field (UDF) has been replaced with custom field in the user interface. However, the API resource is still called UDF. There are two types of custom fields:

- Master step fields—Configured on master steps. Master step fields only apply to the following: The master step on which the fields are configured.The steps derived from those master steps.
- Global fields—Configured on entities (eg, submitted sample, derived sample, measurement, etc.). Global fields apply to the entire Clarity LIMS system.

Activate Workflow, Create Project, Add and Assign Samples

The following steps set up Clarity LIMS in preparation for running samples through the Library Prep Validation v2.0.4 and NextSeq 500/550 Sequencing v1.0 workflows.

1. Activate the Workflows

In the Clarity LIMS configuration area, activate the Library Prep Validation v2.0.4 and NextSeq 500/550 Sequencing v1.0 workflows.

2. Create a Project, Add Samples

1. On the Projects and Samples screen, create a project and add samples to it.
2. Assign your samples to the Library Prep Validation v2.0.4 workflow.

Library Prep Validation v2.0.4 Protocol

This single-step protocol models the library prep required to produce normalized libraries that are ready for the NextSeq 500/550 Sequencing v1.0 workflow.

Run Library Prep Validation v2.0.4 Step

1. In Lab View, locate the Library Prep Validation v2.0.4 protocol. You will see your samples queued for the Library Prep Validation v2.0.4 step.

2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Placement screen, move the samples as follows.
   a. In the Placed Samples area on the right, in the container field, scan or enter the barcode of the destination container.
   b. Select the samples from the Samples to be Placed area on the left. Drag them over to the container wells on the right.
   c. Select Add Labels.
5. On the Add Labels screen, assign the labels as follows.
   a. Select labels on the left and drag them over to the container on the right to assign them to placed samples.
   b. Select Record Details.
6. On the Record Details screen, set the samples to NextSeq as follows.
   a. Select all of the samples in the Sample Details table. From the Sequencing Instrument drop-down list, select NextSeq.
   b. Select Apply to set this value for all selected samples.
   c. Select Next Steps.
7. On the Assign Next Steps screen, the next step is already set to Remove from Workflow. This is required by the Routing script automation.
8. Select Finish Step.
   The Routing script automation is triggered and samples are automatically routed to the first step in the NextSeq 500/550 Sequencing v1.0 protocol. This is the Library Pooling (NextSeq 500/550 v1.0) step.

NextSeq 500/550 Sequencing v1.0 Protocol

1. Run Library Pooling (NextSeq 500/550 v1.0) Step

1. In Lab View, locate the NextSeq 500/550 Sequencing v1.0 protocol. You will see your samples queued for the Library Pooling (NextSeq 500/550 v1.0) step. Select the step to proceed to the Queue screen.
2. On the Queue screen, add samples as follows.
   a. Add the samples to the Ice Bucket. In the Add Control Samples panel, add the PhiX v3 control sample to the Ice Bucket.
   b. Select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Pool Samples screen, create a pool of samples as follows.

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a. Drag samples into the Pool Creator.
b. Name the pool. You can also accept the default name Pool #1.
c. Select Place Samples.

5. On the Placement screen, move the pool to the container as follows.
   a. Select the pool in the Samples to be Placed area on the left. Drag it over to the container on the right.
   b. Select Record Details.
6. On the Record Details screen, select Next Steps.
   On the Assign Next Steps screen, the next step is already set to Denature and Dilute (NextSeq 500/550 v1.0).
7. Select Finish Step.

2. Run Denature and Dilute (NextSeq 500/550 v1.0) Step

1. In Lab View, locate the NextSeq 500/550 Sequencing v1.0 protocol. You will see your pooled samples queued for the Denature and Dilute (NextSeq 500/550 v1.0) step. Select the step to proceed to the Queue screen.
2. On the Queue screen, add the pool to the Ice Bucket. Select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Placement screen, move the pool to the reagent cartridge as follows.
   a. Scan the NextSeq reagent cartridge barcode into the NextSeq Reagent Cartridge field.
   b. Place the pool of samples into the reagent cartridge.
   c. Select Record Details.
5. On the Record Details screen, create the sample sheet as follows.
   a. Under Reagent Lot Tracking, select the reagent lot used in the step. You may need to add/activate the lot on the Reagents and Controls screen.
   b. In the Step Details table, populate the fields as appropriate. The Workflow and Read 1 Cycles are required fields.
   c. In the Samplesheet Template drop-down list, the reverse complement template is selected by default. Do not change this value.
   d. In the Sample Details table, enter the Final Loading Concentration. You may select preset 225 (for PCR-free workflows) or preset 400 (for Nano workflows). You can also enter a different value.
   e. Select Generate bcl2fastq2 NextSeq SampleSheet. Clarity LIMS generates the sample sheet and attaches it and a log file to placeholders in the Files area of the Record Details screen.
   f. Download the files and validate their format and content.
   g. Select Next Steps.

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6. On the Assign Next Steps screen, make sure samples are already assigned to the NextSeq 500/550 Run (NextSeq 500/550 v1.0) step.
7. Select Finish Step.

3. Run NextSeq 500/550 Run (NextSeq 500/550 v1.0) Step

1. In Lab View, locate the NextSeq 500/550 Sequencing v1.0 protocol. You will see your pool of samples queued for the NextSeq 500/550 Run (NextSeq 500/550 v1.0) step. Select the step to proceed to the Queue screen.
2. Add the pool to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
   On the Record Details screen, the fields in the table are read-only. Once the run completes, the integration will automatically populate the fields.
4. Attach files to the Illumina Run Report, Link to Run Folder, Run Parameters, and Run Info placeholders.
   The Log File is attached once the next step for samples has been assigned by the Next Step - Advance automation. Do not continue to step 6 and complete the step until the Illumina Run Report has been attached.
5. Populate the fields in the Sample Details table. See *NextSeq 500/550 Integration v2.2 User and Configuration Guide* for details.
6. Select Next Steps.
   On the Assign Next Steps screen, make sure the next step is already set to Mark protocol as complete.
7. Select Finish Step.

Validating Creation of Event Files

Follow the steps below to confirm that event files are created by the batch file in the destination path. The steps assume that the default configuration has been successfully imported, and support for NCS v4.0 has been configured.

This test validates that:

- Your DESTINATION_PATH is correctly configured.
- The instrument computer can access and write to the DESTINATION_PATH.
- There are no syntax errors in the Clarity LIMS batch file.

You can validate the gls_event_ncs_rta.bat batch file by executing it manually as follows.

1. Edit `gls_event_ncs_rta.bat` using any text editor (Notepad++ recommended).
   By default, this file is installed to `C:\gls`.
2. In the code line set `DESTINATION_PATH=C:\gls\Events\`, change `C:\gls\Events\` to the network path of the event files directory.
   Remember to include the trailing "\" at the end of the file path.

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3. Save your changes.
   Manually executing the batch file will create a dummy EndRun event file in the directory that you defined in the previous step.

Troubleshooting

If an automation trigger does not appear to run its corresponding scripts, see the following topics:

- *Troubleshooting Automation Worker* in the *Administration* section of the *Clarity LIMS v6 Core Functionality* documentation.
- *Troubleshooting Automation* in the *Automation* section of the *Clarity LIMS API & Database* documentation.

If an error occurs that does not provide direction on how to proceed, confirm the version of the installed Illumina NextSeq Integration Package by running the `rpm -qa | grep -i nextseq` command from the server console.

If the error is related to data parsing, retrieving run results data, or report values not appearing as expected, review the `NextSeqIntegrator.log` file. It is located at `/opt/gls/clarity/extensions/Illumina_NextSeq/v2/SequencingService`.

Additional troubleshooting information for this integration is provided on the *Illumina Instrument Integrations FAQ* on page 2598 page.

If you are unable to resolve the issue, contact the Clarity LIMS Support team. Make sure you supply the relevant information from the troubleshooting that was already performed.

NextSeq 500/550 Integration v2.2 Release Notes

These Release Notes describe the key changes to software components for the Clarity LIMS NextSeq 500/550 Integration Package since version 2.1.

This is an optional software update for customers interested in the features available with this release.

If upgrading from a version prior to version 2.1, review the release notes for v2.1 for a list of features and bug fixes introduced in that version.

Compatibility

This version of the integration package is compatible with the following software:

- Clarity LIMS v5.1 and later
- NGS Extensions Package v5.21 and later
- Illumina Preset Protocols (IPP) v2.3.0 and later
- NextSeq Control Software (NCS) v4.0 (Standalone mode only)

New Features

Features provided with this release of the integration package are as follows:

- Support for Clarity LIMS v6.0. Clarity LIMS v4.x and v5.0 are not supported.

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- Fixes to address the following Apache log4j vulnerabilities:
  - CVE-2021-44228
  - CVE-2021-45046
  - CVE-2021-45105
  - CVE-2021-44832
- Tech refresh to upgrade third party libraries.

Defect Repairs

- Fixed run report so that it displays footer correctly.
- Fixed NextSeq sequencing service to record interopUtil parsing in the log file.

Known Issues

None

NextSeq 500/550 Integration v2.1 Configuration and User Interaction

The Illumina NextSeq 500/550 Integration Package v2.1 supports the integration of Clarity LIMS to Illumina NextSeq 500 and 550 sequencing systems.

The integration allows for automated tracking of an Illumina sequencing run in Clarity LIMS, which includes tracking instrument run status, generating run report, and capturing and parsing run statistics. In addition, this integration provides automated generation of a sample sheet file for use with bcl2fastq2 v2.20.0 analysis software.

This document describes the integration between Clarity LIMS and the Illumina NextSeq system. It includes information about protocols and automations, configuration options, installed components, and rules and constraints.

See *NextSeq 500/550 v2.0.3 Validation and Troubleshooting Guide* for instructions on validating and troubleshooting the NextSeq 500/550 Integration Package.

Integration Overview

The Illumina NextSeq 500/550 Integration Package v2.1 includes the following items:

- Automated generation of a sample sheet for use with bcl2fastq2 v2.20.0 analysis software.
- Automated tracking of the NextSeq sequencing run and parsing of run statistics into Clarity LIMS, including:
  - Run status and metrics of sequencing run
  - Sequencing run parameters
  - Real-Time Analysis v2 (RTA2) run directory location and other run-specific information

Prerequisites and Assumptions

It is assumed that samples enter the NextSeq 500/550 Sequencing v1.0 workflow as normalized libraries and have reagent labels attached.

That is, before they are assigned to the workflow:

- Samples have been accessioned into Clarity LIMS.
- Samples have been run through QC and library prep.
- Samples have been normalized, and the value is captured in a field called Normalized Molarity (nM).

For details on sample accessioning, see the following documentation:

- *Sample Accessioning*
- *Upload and Modify Samples*

Samples can be assigned to the NextSeq 500/550 Sequencing v1.0 workflow automatically (using a routing script) or manually from the Projects and Samples screen (see *Assign and Process Samples*).

Workflows, Protocols, and Steps

The Illumina NextSeq Integration includes the NextSeq 500/550 Sequencing v1.0 workflow, which contains a single protocol of the same name.

The NextSeq 500/550 Sequencing System v1.0 protocol includes the following steps:

- Library Pooling (NextSeq 500/550 v1.0)
- Denature & Dilute (NextSeq 500/550 v1.0)
- NextSeq 500/550 Run (NextSeq 500/550 v1.0)

Step Automations

The following table lists the automations configured on the NextSeq 500/550 Sequencing v1.0 steps. Automation details are provided in the configuration details sections following the table. See *NextSeq 500/550 v2.0.3 Validation and Troubleshooting Guide* for details on the validation workflow.

Table 394 NextSeq 500/550 Sequencing System v1.0 Protocol Step Automation

Step	Automation
4. Library Pooling (NextSeq 500/550 v1.0)	• Set Next Step - Advance
5. Denature and Dilute (NextSeq 500/550 v1.0)	• Generate bcl2fastq2 NextSeq 500/550 SampleSheet • Set Next Step - Advance
6. NextSeq 500/550 Run (NextSeq 500/550 v1.0)	• Set Next Step - Advance

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Configuration Details

The following sections describe the configuration installed with the Illumina NextSeq Integration.

The Illumina NextSeq 500/550 Integration Package v2.1 supports Clarity LIMS v4.2, v4.3 and v5.x.

As of Clarity LIMS v5, the term user-defined field (UDF) has been replaced with global custom field in the user interface (the API resource is still called UDF). There are two types of custom fields:

- Master step fields—Configured on master steps. These fields only apply to the master step on which they are configured and the steps derived from those master steps.
- Global fields—Configured on entities such as submitted sample, derived sample, measurement, etc. These fields apply to the whole Clarity LIMS system.

Step 1: Library Pooling (NextSeq 500/550 v1.0)

In Clarity LIMS v5, the Library Pooling (NextSeq 500/550 v1.0) step is derived from the Library Pooling v1.0 master step. Libraries are placed into pools manually.

Automations

The following automation is configured on the Library Pooling (NextSeq 500/550 v1.0) step:

- Set Next Step - Advance

Set Next Step - Advance

This automation is automatically triggered on exit of the Record Details screen. This automation advances samples to the next step in the protocol. The following command line is the default:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-t false \
-h false \
-exp 'nextStep = ::ADVANCE::' \
-log {compoundOutputFileLuid0}"
```

Step UDFs/Custom Fields

This field is configured on the Library Pooling (NextSeq 500/550 v1.0) step and displays on the Record Details screen at run time.

The following table shows field configuration details.

Table 395 Library Pooling (NextSeq 500/550 v1.0) Step Custom Field Configuration

Field Name	Field Type	Field Constraints/Options

Comment	Multiline Text	• Required: No • Read Only: No

Global Custom Fields

The following table lists the global custom fields configured to display on the Queue and Ice Bucket screens of the Library Pooling (NextSeq 500/550 v1.0) step. Most of these fields show in the expanded view only.

Table 396 Global Custom Field Configuration for Library Pooling (NextSeq 500/550 v1.0)

Field Name	Field Name	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Configured on Sample in Clarity LIMS v4.2 and Submitted Sample in Clarity LIMS v5			

Application	Single-line text (v4.2) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: Yes	• TruSeq mRNA sequencing • TruSeq DNA sequencing (large genome de novo) • TruSeq DNA sequencing (large genome reseq) • TruSeq DNA sequencing (small genome de novo) • TruSeq DNA sequencing (small genome reseq) • Nextera DNA sequencing • TruSeq Custom Amplicon sequencing • ChIP-sequencing • Exome sequencing • Mate pair sequencing • Small RNA sequencing
Pooling	Single-line text (v4.2) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: Yes	• Yes • No
Read Length	Single-line text (v4.2) Text (v5)	• Required: No • Read Only: No	Not applicable
Sequencing Coverage	Single-line text (v4.2) Text (v5)	• Required: No • Read Only: No	Not applicable

Sequencing Method	Single-line text (v4.2) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: Yes	• Single Read • Paired End Read • Indexed Single read • Indexed Paired End Read
Configured on Analyte in Clarity LIMS v4.2 and Derived Sample in Clarity LIMS v5			
Normalized Molarity (nM)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2

User Interaction

1. On the Queue screen, add the samples to the Ice Bucket.
2. In the Add Control Samples panel, add the PhiX v3 control sample to the Ice Bucket.
3. Select View Ice Bucket.
4. On the Ice Bucket screen, select Begin Work.
5. On the Pool Samples screen, create a pool of samples by dragging samples into the Pool Creator. Name the pool or accept the default name (Pool #1).
6. Select Place Samples.
7. On the Placement screen, select the pool in the Samples to be Placed area on the left and drag it to the container on the right.
8. Select Record Details.
9. On the Record Details screen, select Next Steps.
10. On the Assign Next Steps screen, next step is set to Denature and Dilute (NextSeq 500/500 v1.0).
11. Select Finish Step.

Step 2: Denature and Dilute (NextSeq 500/550 v1.0)

In this step, pooled libraries are denatured and diluted and placed into the reagent cartridge that is loaded into the NextSeq instrument.

Automations

The following automations are configured on the Denature and Dilute (NextSeq 500/550 v1.0) step:

- Generate bclfastq2 NextSeq SampleSheet
- Set Next Step - Advance

Generate bcl2fastq2 NextSeq SampleSheet

This automation is triggered by a button on the Record Details screen.

This automation generates the sample sheet and attaches it to the step. For details, see the following section on sample sheet generation. The following command line is the default:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {processURI:v2} -u {username} -p
{password} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/{udf:Samplesheet
Template} \
-o {compoundOutputFileLuid1}.csv \
-q true \
-destLIMSID {compoundOutputFileLuid1} \
-l {compoundOutputFileLuid2}"
```

Set Next Step - Advance

This automation is automatically triggered on exit of the Record Details screen.

This automation advances samples to the next step in the protocol. In Clarity LIMS v4.2, the automation also invokes the setUDF script, which sets the value of the Progress UDF. Clarity LIMS updates this UDF value as samples progress through the workflow steps. The Progress UDF does not display in the Clarity LIMS user interface. However, it does display in the LabLink Collaborations Interface so that collaborators can see the status of their samples.

For Clarity LIMS v4.2, the following command line is the default:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -u {username} -p {password} \
script:setUDF \
-i {processURI:v2} \
-f 'Progress' \
-t '//input/@uri->//sample/@uri' \
-v 'Library ready for sequencing' \
script:evaluateDynamicExpression \
-i {stepURI:v2:http} \
-t false \
-h false \
-exp 'nextStep = ::ADVANCE::' \
-log {compoundOutputFileLuid3}"
```

For Clarity LIMS v5, the following command line is the default:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-t false \
```

```
-h false \
-exp 'nextStep = ::ADVANCE::' \
-log {compoundOutputFileLuid3}"
```

Step UDFs/Custom Fields

There are 10 fields configured on the Denature and Dilute (NextSeq 500/550 v1.0) step. At run time, these fields display on the Record Details screen, in the Step Data table. The fields are manually populated. Their values are used to generate the sample sheet.

The following table lists field configuration details.

Table 397 Denature and Dilute (NextSeq 500/550 v1.0) Step Custom Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Adapter	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: No	Not applicable
Adapter Read 2	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: No	Not applicable
Experiment Name	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: No	Not applicable
Mask Adapter	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: No	Not applicable
Mask Adapter Read 2	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: No	Not applicable
Read 1 Cycles	Numeric (v4.2) Numeric Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: Yes	• 251 (Default) • 151 • 101 • 76 • 51 • Range From: 0 to 1000 • Decimal Places Displayed: 0

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Read 2 Cycles	Numeric (v4.2) Numeric Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: Yes	• 251 • 151 • 101 • 76 • 51 • Range From: 0 to 1000 • Decimal Places Displayed: 0
SampleSheet Template	Single-line Text (v4.2) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: No	• BCL2FASTQ_ Reverse_ Complement_ Samplesheet.csv (default) • BCL2FASTQ_ Samplesheet.csv
Workflow	Single-line Text (v4.2) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: No	• Assembly • Custom Amplicon • Enrichment • GenerateFASTQ • LibraryQC • Metagenomics • PCR Amplicon • Resequencing

Groups of Defaults/Presets

The following group of defaults/presets are configured for Denature and Dilute (NextSeq 500/550 v1.0) master step custom fields:

- Resequencing
  - Read 1 Cycles: 251
  - Workflow: Resequencing
- Custom Amplicon
  - Read 1 Cycles: 251
  - Workflow: Custom Amplicon
- PCR Amplicon
  - Read 1 Cycles: 251
  - Workflow: PCR Amplicon
- Enrichment

  - Read 1 Cycles: 251
  - Workflow: Enrichment
- Library QC
  - Read 1 Cycles: 251
  - Workflow: LibraryQC
- Metagenomics
  - Read 1 Cycles: 251
  - Workflow: Metagenomics
- GenerateFastQ
  - Read 1 Cycles: 251
  - Workflow: GenerateFastQ
- Assembly
  - Read 1 Cycles: 251
  - Workflow: Assembly

Global Custom Fields

The following table lists the global custom fields that are configured to display on the Queue, Ice Bucket, and Record Details screens of the Denature and Dilute (NextSeq 500/550 v1.0) step.

Table 398 Global Custom Field Configuration for Denature and Dilute (NextSeq 500/550 v1.0) Step

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-down Items
Configured on Sample in Clarity LIMS v4.2 and Submitted Sample in Clarity LIMS v5			
Progress (Clarity LIMS v4.2) Displays in LabLink Collaborations Interface only	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: No • Custom Entries: Yes	Not applicable
Read Length Displays on Queue & Ice Bucket screens	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: No	Not applicable

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Sequencing Method Displays on Queue & Ice Bucket screens	Single-line Text (v4.2) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: Yes	• Single Read • Paired End Read • Indexed Single Read • Indexed Paired End Read
Configured on Analyte in Clarity LIMS v4.2 and Derived Sample in Clarity LIMS v5			
Final Loading Concentration Displays on Record Details screen	Numeric (v4.2) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 225 • 400

Step File Placeholders

Placeholders for the following files are configured on the Record Details screen of the Denature and Dilute (NextSeq 500/550 v1.0) step:

- Lab Tracking Form (manually generated)
- bcl2fastq2 Samplesheet (autogenerated)
- bcl2fastq2 Samplesheet Generation Log (autogenerated)
- Log File (autogenerated)

Table 399 Sample Sheet Files & Placeholders Generated by Denature & Dilute (NextSeq 500/550 v1.0) Step

Item	Description
Lab Tracking Form placeholder	This placeholder in Clarity LIMS allows for manually attaching a lab-specific tracking form to the step.
bcl2fastq2 SampleSheet	This CSV file is automatically generated by Clarity LIMS for use with the bcl2fastq2 v2.20.0 analysis software. It can be opened as a text file or as an MS Excel spreadsheet.
bcl2fastq2 SampleSheet Generation Log	Automatically generated by Clarity LIMS, this log file captures any errors that Clarity LIMS can encounter when generating the sample sheet.
Log File	Automatically generated by Clarity LIMS, this log file captures the status of the EvaluateDynamicExpression script that is launched by the Set Next Step Advance automation.

User Interaction

1. On the Queue screen, add the pool to the Ice Bucket and select View Ice Bucket.
2. On the Ice Bucket screen, select Begin Work.

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3. On the Placement screen, scan the barcode of the NextSeq reagent cartridge into the NextSeq Reagent Cartridge field, then place the pool of samples into the reagent cartridge.
4. Select Record Details.
5. On the Record Details screen under Reagent Lot Tracking, select the reagent lot used in the step. (Add/activate the lot on the Reagents and Controls screen, if necessary.)
6. Populate the fields in the Step Details table. Workflow and Read 1 Cycles are required fields.
7. In the Sample Details table, enter a Final Loading Concentration value. Select from the two preset options, 225 (for PCR-free workflows) or 400 (for Nano workflows), or enter a different value manually.
8. Select Generate bcl2fastq2 NextSeq SampleSheet.
9. Clarity LIMS generates the samplesheet and attaches it and a log file to placeholders in the Files area of the Record Details screen. Download the files and validate their format and content.
10. Select Next Steps.
    On the Assign Next Steps screen, samples are assigned to the NextSeq 500/550 Run (NextSeq 500/550 v1.0) step.
11. Select Finish Step.

Step 3: NextSeq 500/550 Run (NextSeq 500/550 v1.0)

In this step, pooled samples are sequenced on the NextSeq 500/550 instrument and the run metrics are recorded in Clarity LIMS

Automations

The following automation is configured on the NextSeq 500/550 Run (NextSeq 500/550 v1.0) step:

- Set Next Step - Advance

Set Next Step - Advance

This automation is automatically triggered on exit of the Record Details screen, and advances samples to the next step in the protocol. The default commend line is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-t false \
-h false \
-exp 'nextStep = ::ADVANCE::' \
-log {compoundOutputFileLuid5}"
```


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Step UDFs/Custom Fields

There are 16 fields configured on the NextSeq 500/550 Run (NextSeq 500/550 v1.0) step. These fields display on the Record Details screen at run time. Some of the field values must be completed manually, and the remaining fields are automatically populated at the end of the run.

The following table lists field configuration details.

Table 400 NextSeq 500/550 Run (NextSeq 500/550 v1.0) Step Custom Field Configuration

Field Name	Field Type	Filed Constraints/Options	Preset Value/Additional Options and Drop-down Items
Chemistry	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: Yes	Not applicable
Comment	Multiline Text	• Required: No • Read Only: No	Not applicable
Experiment Name	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: Yes	Not applicable
Finish Date	Not applicable	• Required: No • Read Only: Yes	Not applicable
Flow Cell ID	Not applicable	• Required: No • Read Only: Yes	Not applicable
Index 1 Read Cycles	Numeric	• Required: No • Read Only: Yes	Decimal Places Displayed: 0
Index 2 Read Cycles	Numeric	• Required: No • Read Only: Yes	Decimal Places Displayed: 0
Output Folder	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: Yes	Not applicable
PR2 Bottle ID	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: Yes	Not applicable
Read 1 Cycles	Numeric	• Required: No • Read Only: Yes	Decimal Places Displayed: 0
Read 2 Cycles	Numeric	• Required: No • Read Only: Yes	Decimal Places Displayed: 0

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Reagent Cartridge ID	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: Yes	Not applicable
Run ID	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: Yes	Not applicable
Run Type	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: Yes	Not applicable
Status	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: Yes	Not applicable
Workflow	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: Yes	Not applicable

Global Custom Fields

There are several sample and measurement global custom fields configured to display on the Record Details screen of the NextSeq 500/550 Run (NextSeq 500/550 v1.0) step. These fields are populated at the end of the sequencing run.

Step File Placeholders

Placeholders for the following files are configured on the Record Details screen of the NextSeq 500/550 Run (NextSeq 500/550 v1.0) step.

- Illumina Run Report (autogenerated)
- Link to Run Folder (autogenerated)
- Run Parameters (autogenerated)
- Run Info (autogenerated)
- Lab Tracking Form (manually generated)
- Log File (autogenerated)

User Interaction

1. On the Queue screen, add the pool to the Ice Bucket and select View Ice Bucket.
2. On the Ice Bucket screen, select Begin Work.
   On the Record Details screen, the fields in the table are read-only. After the run completes, the integration automatically:
   - Populates the fields.
   - Attaches files to the Illumina Run Report, Link to Run Folder, Run Parameters, and Run Info placeholders.

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- Populates the fields in the Sample Details table.

3. Select Next Steps.
4. On the Assign Next Steps screen, the next step is set to Mark protocol as complete.
5. Select Finish Step.

Sample Sheet Generation

Sample sheet generation occurs on the step before the sequencing run Denature and Dilute (NextSeq 500/550 v1.0). Samples are placed on the container to be loaded in the instrument. The default configuration provides one automation, Generate bcl2fastq2 NextSeq SampleSheet. This automation uses the Template File Generator (DriverFileGenerator.jar) and a template file to generate a CSV format file for use with bcl2fastq2 v2.20.0 analysis software. The sample sheet content is determined by the fields that display on the Record Details screen of the step (in the Step Data table) and the values entered into these fields. Templates can be customized to create the sample sheet and insert additional columns if the lab desires.

Sequencing Results Parsing

The NextSeq 500/550 Run (NextSeq 500/550 v1.0) step records information for the flow cell lanes and generates a report summarizing the results. In addition, run parameters, run info, and a link to the run folder are automatically captured.

Generated and Captured Files

The following table lists the run information files, reports, placeholders, and links that Clarity LIMS automatically generates or capture during a sequencing run.

Table 401 Run Information Generated or Captured by NextSeq 500/550 Run (NextSeq 500/550 v1.0) Step

Item	Description
Run Info Run Parameters	These XML files are captured automatically by Clarity LIMS from the instrument run folder. They include the key run parameters, many of which are parsed out into key step global custom fields.
Link to Run Folder	Automatically generated by Clarity LIMS, and is a link to the network run folder where the data that was captured from the instrument during the run is stored.

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Illumina Run Report	Automatically generated by Clarity LIMS, this report provides key information about the run and the samples on the flow cell. Information includes the flow cell ID, run directory location, and primary analysis metrics for the instrument, summarized per flow cell lane for the entire run, and individual reads if there are paired-end runs. These metrics are compared against the instrument per lane averages, calculated using metrics from the last 5 sequencing runs. Any values outside of 1 standard deviation are highlighted.
Lab Tracking Form placeholder	This placeholder in Clarity LIMS allows you to attach a lab-specific tracking form to the step manually.

Metadata

The following list includes the metadata that Clarity LIMS automatically captures from the Illumina sequencing software as part of a sequencing run. This information is gathered from various run result files and events.

- Chemistry
- Experiment Name – entered in software
- Finish Date*—Run completion date
- Flow Cell ID
- Index 1 Read Cycles—Intended Index cycles
- Index 2 Read Cycles—Intended Index cycles
- Output Folder—Run folder root
- PR2 Bottle ID
- Reagent Cartridge ID
- Read 1 Cycles
- Read 2 Cycles
- Run ID—The unique run ID
- Run Type
- Status—Current status of the sequencing run on the instrument
- Workflow

If the End Run event contains a date in the format YYYY-MM-DD, Finish Date is set to the date in the event file.

If the End Run event does not contain a date or the date is in the wrong format, Finish Date is set to the date when the event file is processed.

Primary Analysis Metrics

The following table lists the Real-Time Analysis v2 (RTA2) primary analysis metrics that Clarity LIMS automatically captures and records, per read, for samples in each flow cell lane. These metrics are captured after run completion and are stored as global custom fields in the Record Details screen Sample Details table. Per read and per lane metrics are viewable by expanding the output.

Table 402 RTA Primary Analysis Metrics Captured by NextSeq 500/550 Run (NextSeq 500/550 v1.0) Step

Per Read Clarity LIMS Field Name (stored on derived sample/analyte input to the step)	Per Lane Clarity LIMS Field Name (stored in measurement placeholders in Record Details screens Sample Details table)
% Aligned R1	% Aligned R1
% Aligned R2	% Aligned R2
% Bases >=Q30 R1	% Bases >=Q30 R1
% Bases >=Q30 R2	% Bases >=Q30 R2
% Error Rate R1	% Error Rate R1
% Error Rate R2	% Error Rate R2
	% Phasing R1
	% Phasing R2
	% Prephasing R1
	% Prephasing R2
	%PF R1
	%PF R2
	Cluster Density (K/mm^2) R1
	Cluster Density (K/mm^2) R2
Intensity Cycle 1 R1	Intensity Cycle 1 R1
Intensity Cycle 1 R2	Intensity Cycle 1 R2
	Reads PF (M) R1
	Reads PF (M) R2
Yield PF (Gb) R1	Yield PF (Gb) R1
Yield PF (Gb) R2	Yield PF (Gb) R2

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The sequencing service runs on the Clarity LIMS server. The service detects event files that the instrument software (RTA2) produces as the run progresses, which tells the service where to find the run data. As the run data is written out and the End Run event is detected, the data is matched to the step. This matching is based on the reagent cartridge ID that was entered/scanned in the Denature and Dilute (NextSeq 500/550 v1.0) step. Read-only field values on the Record Details screen are populated accordingly. When finished processing the end run event and updating the fields in Clarity LIMS, the sequencing service generates the report and attaches it to the step.

User Interaction

1. Begin the step process and wait until the sequencing service has finished processing the run and the run report is generated and attached.
2. On the Record Details screen, the read-only field values are automatically populated as the instrument runs.
   After the run has completed:
   - The read-only fields are populated.
   - The Illumina Run Report is generated and attached.
   - The Link to Run Folder is generated and attached.
   - The Run Parameters and Run Info files are attached.
3. [Optional] In the Files section, upload the Lab Tracking Form.
   The steps complete. The Log File is attached after the next step for samples is assigned by the Next Step, Advance automation.

   Do not complete the step until the Illumina Run Report is attached to the step in Clarity LIMS.

Components Installed

This integration requires installation of the Preconfigured Workflow Package (PCWP).

The following table lists the scripts and files installed in the Illumina NextSeq 500/550 Integration Package v2.1 RPM. Additional component details are provided in the sections following the table.

Table 403 Illumina NextSeq 500/550 Integration Package v2.1 Scripts and Files Installed

Files Installed	Location and Description
configure_extensions_nextseq_sequencingservice.sh	/opt/gls/clarity/config/ Script that installs the service properties in the database.
log4j.xml	/opt/gls/clarity/extensions/nextseq/SequencingService/conf File containing the settings for the sequencing jar logging.
nextseq-sequencing.jar	/opt/gls/clarity/extensions/nextseq/SequencingService Jar file containing API-based Clarity LIMS extensions used for capturing run results and report generation.

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InterOp libraries	/opt/gls/clarity/extensions/nextseq/lib Illumina shared library for parsing InterOp data files.
• BCL2FASTQ_Reverse_ Complement_Samplesheet.csv • BCL2FASTQ_Samplesheet.csv (Installed by the PCWP)	/opt/gls/clarity/extensions/nextseq/Templates Template file used for file generation. In this integration, the reverse complement template is used by default.

It is possible to configure support for multiple, identical seqservice.netPathPrefixSearch property values.

Database Properties Installed

Additional properties, each with the 99 suffix appended to their name, are also installed and intended for use by the Clarity LIMS Support team in automated validation tests. Those properties are not listed in the table.

Constraints

Sequencing runs are matched using the reagent cartridge ID and the sequencing steps base name, NextSeq 500/550 Run (NextSeq 500/550 v1.0).

Do not change the base name. The name is expected by the sequencing service that captures instrument run results. The base name is stored in the sequenceProcessBaseName database property. If this name is changed without the database property being updated, the 'reagent cartridge ID <-> sequencing step base name' matching system fails.

If necessary, modify the step name by editing or adding text after the base name portion, as it is not used in the matching system. For example, change NextSeq 500/550 Run (NextSeq 500/550 v1.0) to NextSeq 500/550 Run (NextSeq 500/550 v1.0) v2.1.

The following table lists the database properties installed with Illumina NextSeq 500/550 Integration v2.1.

Table 404 Database Properties Installed with the Illumina NextSeq 500/550 Integration Package

Property	Description	Default Value
nextseq.v2.seqservice.sequenceProcessBaseName (installed from the PCWP)	Sequencing process type / master step base display name. Partial matching is used to look up the process type / master step.	NextSeq 500/550 Run (NextSeq 500/550 v1.0)

nextseq.v2.seqservice.eventFileDirectory.1	A network location monitored for event files. Eg, /mnt/illumina/gls_events/'	/mnt/gls_events
nextseq.v2.seqservice.netPathPrefixSearch.1	The network directory prefix contained in the event file - most likely in Windows format.	\\nas\network\run_data
nextseq.v2.seqservice.netPathPrefixReplace.1	The mapped network directory mount name on the server used to access the run data directory. Eg. /mnt/network/data'	/mnt/run_data
nextseq.v2.seqservice.eventFileDirectorySuffixes	A list of eventFileDirectory path entries to monitor for event files. The value is one or more comma-separated integers.	99 Configured on install to point to 1.

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nextseq.v2.seqservice.netPathPrefixSearchReplaceSuffixes	A list of netPathPrefix search and replace entries for transforming Windows to Linux network paths. The value is one or more comma-separated integers.	99 Configured on install to point to 1.
nextseq.v2.seqservice.runReportViewsVersion	The current version of the Run Report views in the database. The value 0 represents the state before the views are created. This property is automatically updated by the run report.	0
nextseq.v2.seqservice.ignoreUnmatchedContainerIds	A flag indicating if event files that cannot be matched to reagent cartridges in Clarity LIMS should be archived after a certain time (true), or continually reprocessed (false).	false Note: To prevent the gls_events file directory from becoming cluttered, it is recommended that the value of this property is set to true.

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nextseq.v2.seqservice.ignoreUnmatchedContainerIdsWaitDays	The number of days between when the event is created and the event file is archived.	14
nextseq.v2.seqservice.synchronizationPeriod	Invocation period in seconds.	60

Configure support for multiple, identical seqservice.netPathPrefixSearch property values.

Reagent categories/label groups are installed with the PCWP workflow configuration slices.

The NextSeq Reagent Kit is included in the Illumina NextSeq Integration.

Control types installed.

The PhiX v3 control type is included in the Illumina NextSeq Integration.

The NextSeq Reagent Cartridge container type is included in the Illumina NextSeq Integration.

Instrument Integration

To make sure that the Illumina instrument warranty remains valid, the instrument integration must be performed and maintained by the Clarity LIMS Support team. To perform this integration, the Support team requires remote access to the instrument while it is idle.

To configure the Illumina instrument for use with the Illumina NextSeq Integration, the Support team:

1. Creates a directory on the local computer to hold the batch files. These batch files write event files to the network attached storage (NAS) shares.
2. Creates a directory on the NAS to hold the event files.
3. Modifies Illumina software configuration files to call the batch files that create the event files.
4. Updates sequencing service default database properties to match the specifics of the installation.

All one-dimensional container types with both numeric rows and numeric columns are supported.

This integration operates with the following constraints:

- The reagent cartridge ID must be unique. Avoid multiple reagent cartridge containers in the system with identical names.
- The reagent cartridge ID must be scanned as the reagent cartridge Container Name on the Denature and Dilute (NextSeq 500/550 v1.0) step.

Resources

NextSeq 550 information

NextSeq 500 System Support Resources

NextSeq 550 System Support Resources

NextSeq 500/550 Integration v2.1 Release Notes

These Release Notes detail the key changes to software components for the Clarity LIMS NextSeq 500/550 Integration Package since version 2.0 of that package.

This is an optional software update for customers interested in the new features available with this new release.

If you are upgrading from a version prior to version 2.0 please review the release notes for version 2.0 for a list of features and bug fixes introduced in that version.

Compatibility

- Clarity LIMS v4.2 through v5.4
- NGS Extensions Package v5.19 or later
- Preconfigured Workflow Package (PCWP) v2.0.1 or later
- Illumina Preset Protocols (IPP) v2.0.0 or later
- NextSeq Control Software (NCS) v4.0 (Standalone mode, only)

New Features

This release of the Clarity LIMS integration package:

- Supports the security improvement made to the management of passwords in Clarity LIMS v5.4.
- Provides an updated run report with the Clarity LIMS footer.
- Delivers a NextSeq preconfigured workflow as part of PCWP 2.0.1 or IPP 2.0.0, which can be installed via support request.

Defect Repairs

The SequencerApi proxy configuration script now correctly updates the clarity.conf file.

Known Issues

None

Illumina NextSeq 500/550 Integration v2.0.3 Validation and Troubleshooting

Introduction

This guide explains how to validate the installation of the Illumina NextSeq 500/550 Integration Package v2.0.3.

The validation process involves:

- Running samples through the Library Prep Validation v1.0 workflow. The workflow contains a single-step protocol that models the library prep required to produce normalized libraries. At the end of the step, the normalized libraries are automatically advanced to the NextSeq 500/550 Sequencing v1.0 workflow.
- Running normalized libraries through the NextSeq 500/550 Sequencingv1.0 workflow. This validates the following:
  - Automated generation of a sample sheet for use with bcl2fastq2 v2.20.0 analysis software.
  - Automated tracking of the NextSeq sequencing run and parsing of run statistics into the LIMS, including:
    - Run status and metrics of sequencing run
    - Sequencing run parameters
    - Real Time Analysis v2 (RTA2) run directory location and other run specific information
- Validating creation of event files

Validation Steps

Activate Workflow, Create Project, Add and Assign Samples

The following steps set up the LIMS in preparation for running samples through the Library Prep Validation v1.0 and NextSeq 500/550 Sequencing v1.0 workflows.

1. Activate the Workflows

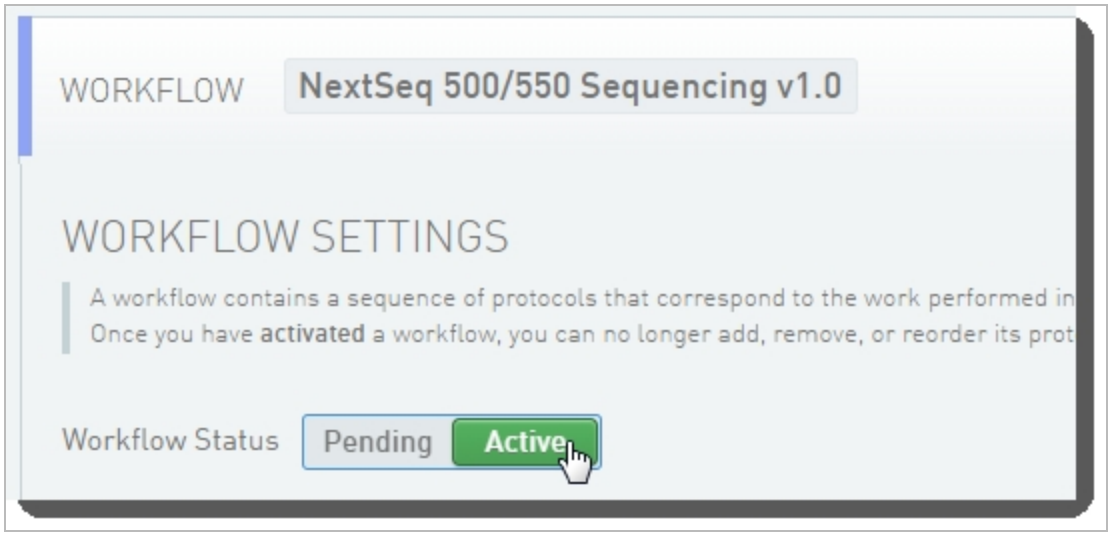
In the LIMS configuration area, activate the Library Prep Validation v1.0 and NextSeq 500/550 Sequencing v1.0 (shown below) workflows.

Figure 1 Workflow configuration and activation - LIMS v4.2



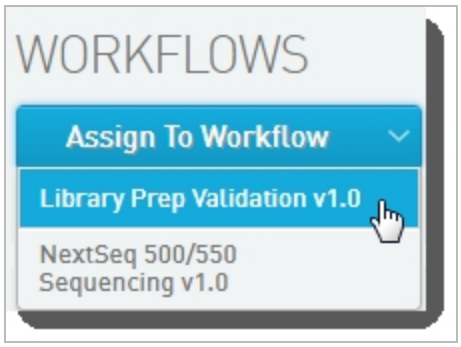
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Figure 2 Workflow configuration and activation - LIMS v5.0



2. Create a Project, Add Samples

1. On the Projects and Samples screen, create a project and add samples to it.
2. Assign your samples to the Library Prep Validation v1.0 workflow.



Library Prep Validation v1.0 Protocol

This single-step protocol models the library prep required to produce normalized libraries that are ready for the NextSeq 500/550 Sequencing v1.0 workflow.

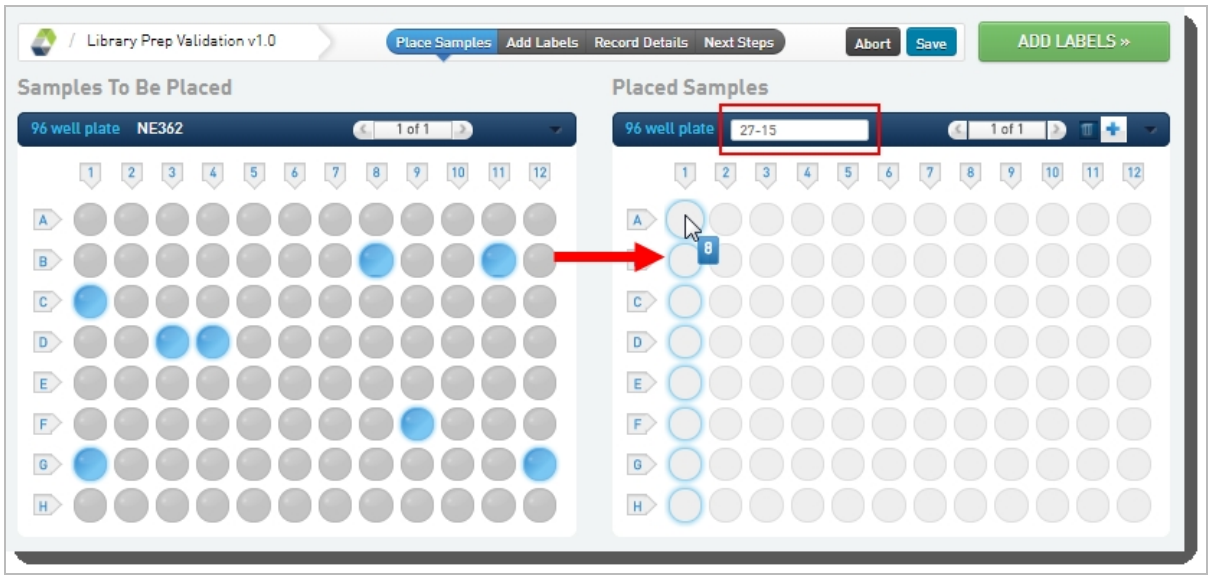
Run Library Prep Validation v1.0 Step

1. In Lab View, locate the Library Prep Validation v1.0 protocol. You will see your samples queued for the Library Prep Validation v1.0 step.

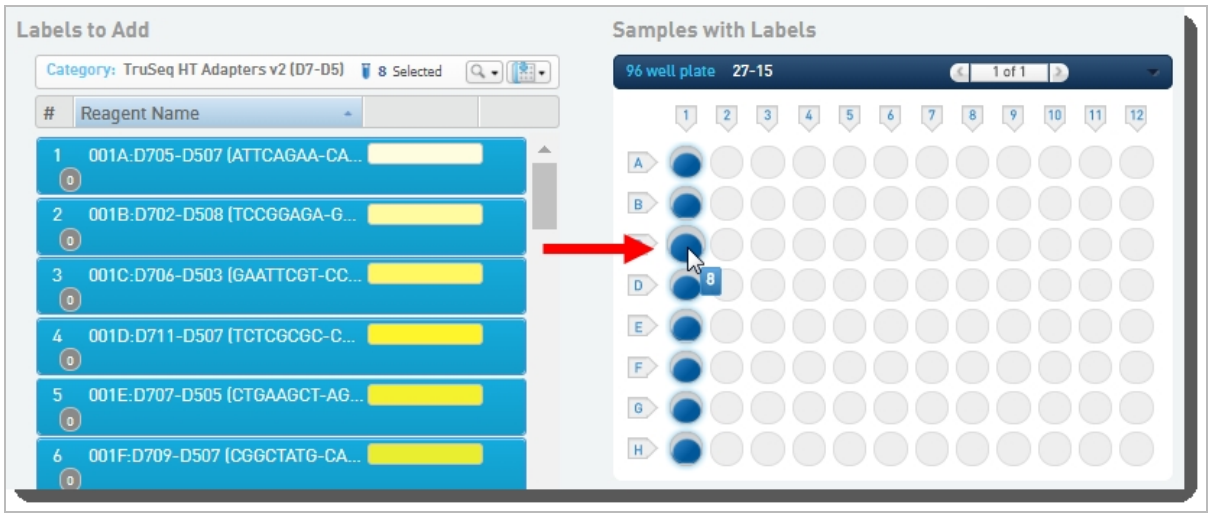


2. Add the samples to the Ice Bucket and click View Ice Bucket.
3. On the Ice Bucket screen, click Begin Work.

4. On the Placement screen:
   a. In the Placed Samples area on the right, in the container field, scan or enter the barcode of the destination container.
   b. Select the samples from the Samples to be Placed area on the left, and then drag them over to the container wells on the right.



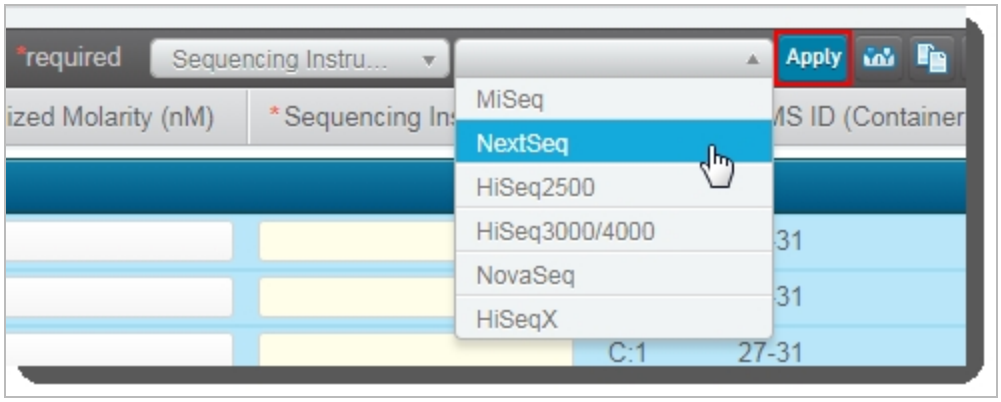
   c. Click Add Labels.
5. On the Add Labels screen:
   a. Select labels on the left, and drag them over to the container on the right to assign them to placed samples.



   b. Click Record Details.
6. On the Record Details screen:

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a. Select all of the samples in the Sample Details table and then in the Sequencing Instrument dropdown list, select NextSeq.



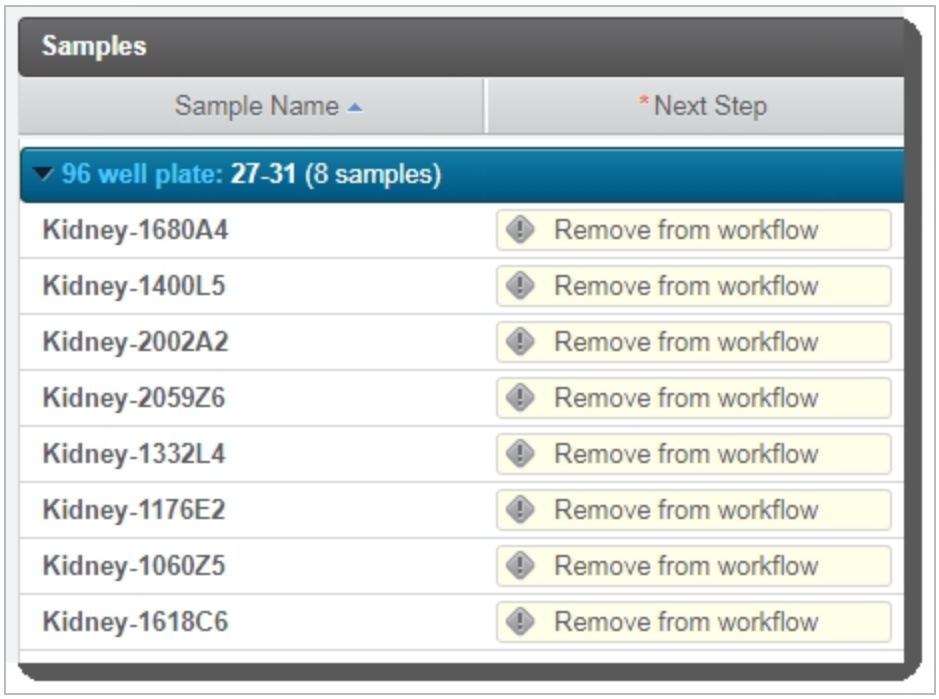
b. Click Applyto set this value for all selected samples.



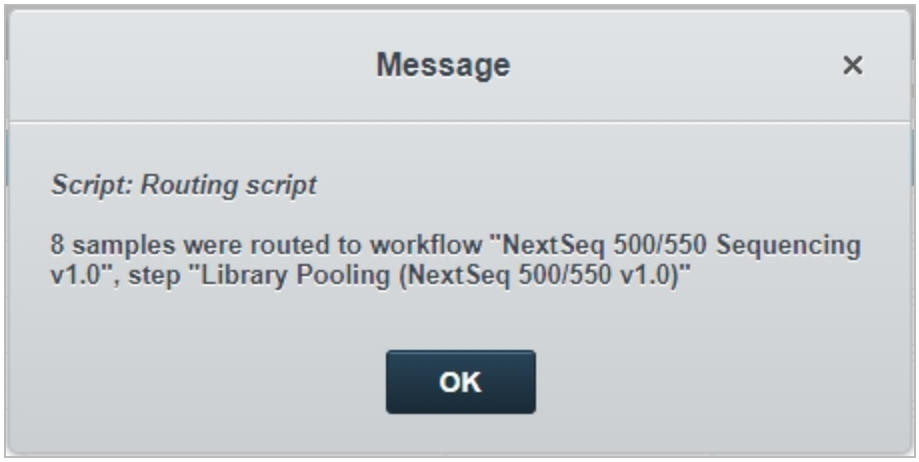
c. Click Next Steps.

7. On the Assign Next Steps screen, the next step is already set to Remove from Workflow (this is required by theRouting scriptautomation).

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8. Click Finish Step.The Routing script automation is triggered and samples are automatically routed to the first step in the NextSeq 500/550 Sequencing v1.0 protocol - Library Pooling (NextSeq 500/550 v1.0).

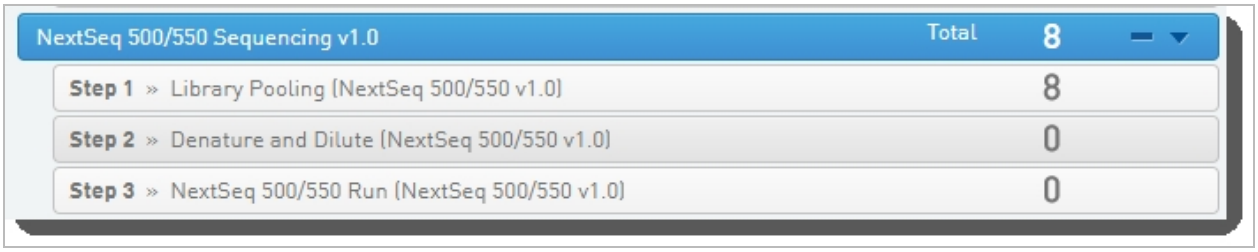


NextSeq 500/550 Sequencing v1.0 Protocol

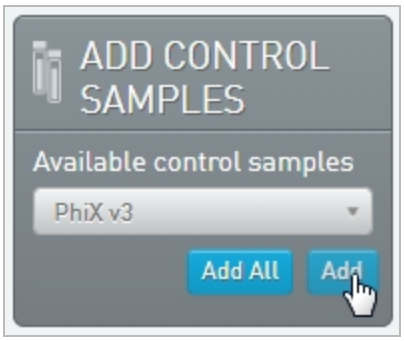
1. Run Library Pooling (NextSeq 500/550 v1.0) Step

1. In Lab View, locate the NextSeq 500/550 Sequencing v1.0 protocol. You will see your samples queued for the Library Pooling (NextSeq 500/550 v1.0) step. Click the step to proceed to the Queuescreen.

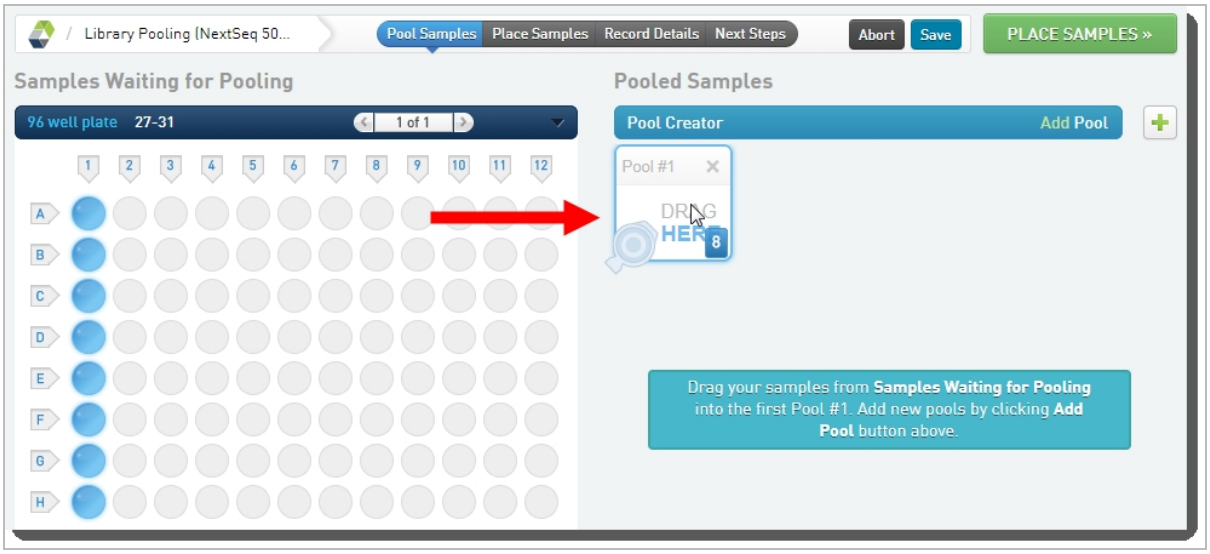
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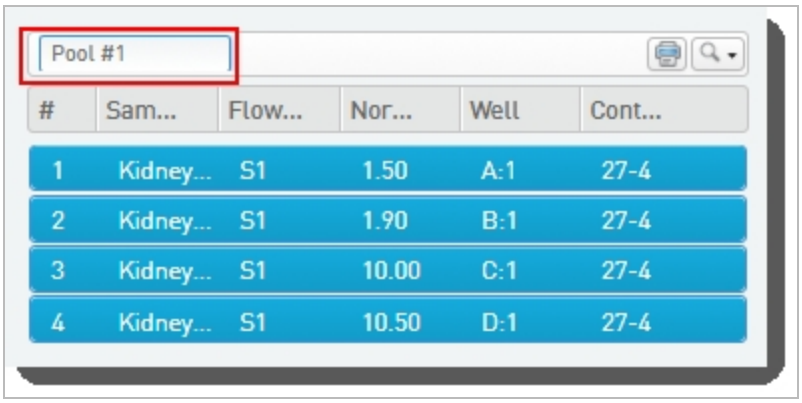
2. On the Queue screen:
   a. Add the samples to the Ice Bucket. In the Add Control Samples panel, add the PhiX v3 control sample to the Ice Bucket.



   b. Click View Ice Bucket.
3. On the Ice Bucket screen, click Begin Work.
4. On the Pool Samples screen:
   a. Create a pool of samples by dragging samples into the Pool Creator.



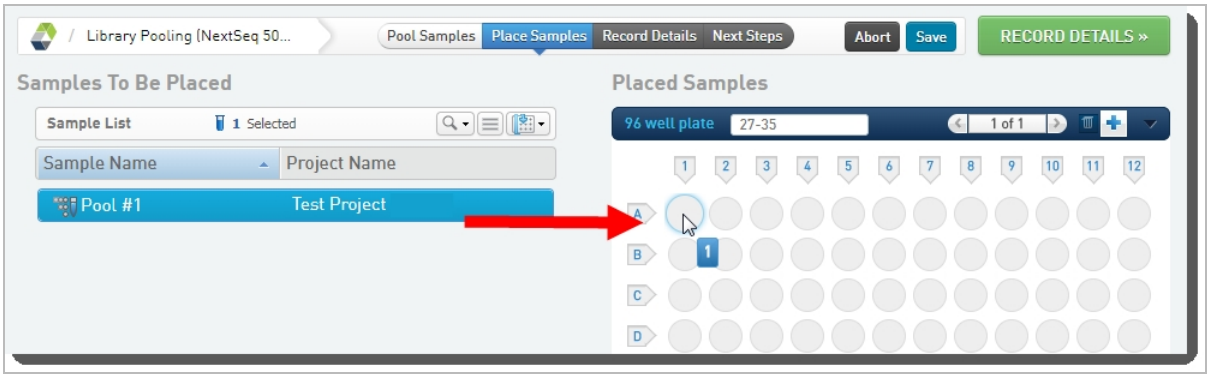
   b. Name the pool, or accept the default name - Pool #1.



c. Click Place Samples.

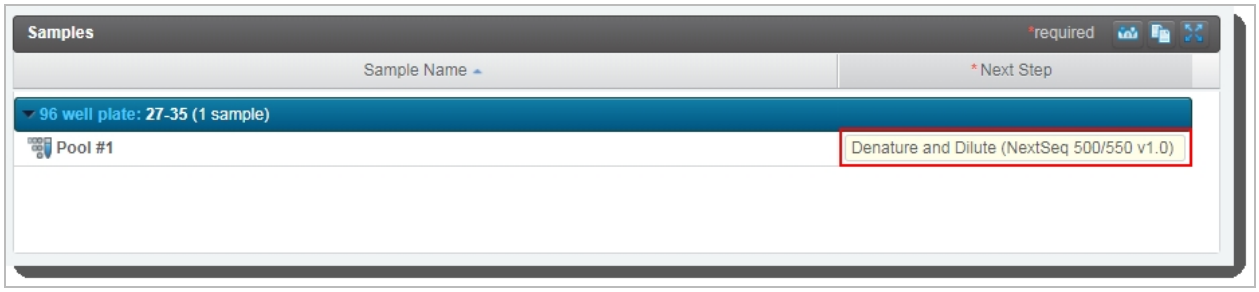
5. On the Placement screen:

a. Select the pool in the Samples to be Placed area on the left and drag it over to the container on the right.



b. Click Record Details.

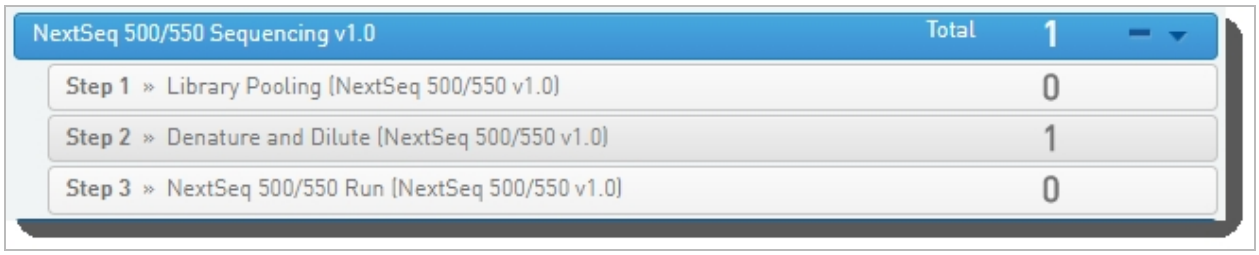
6. On the Record Details screen, click Next Steps.
On the Assign Next Steps screen, next step is already set to Denature and Dilute (NextSeq 500/550 v1.0).



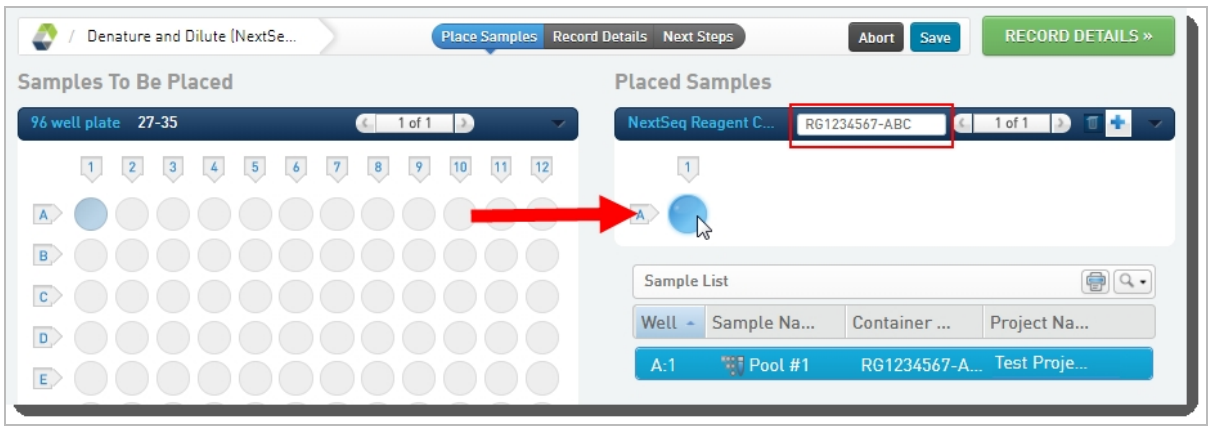
7. Click Finish Step.

2. Run Denature and Dilute (NextSeq 500/550 v1.0) Step

1. In Lab View, locate the NextSeq 500/550 Sequencing v1.0 protocol. You will see your pooled samples queued for the Denature and Dilute (NextSeq 500/550 v1.0) step. Click the step to proceed to the Queuescreen.

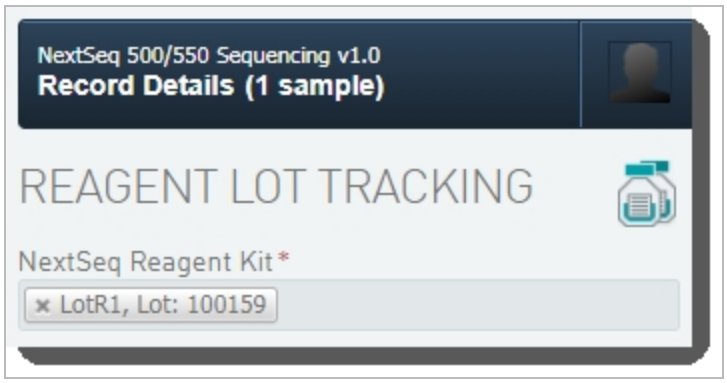


2. On the Queue screen, add the pool to the Ice Bucket and click View Ice Bucket.
3. On the Ice Bucket screen, click Begin Work.
4. On the Placement screen:
   a. Scan the NextSeq reagent cartridge barcode into the NextSeq Reagent Cartridge field.
   b. Place the pool of samples into the reagent cartridge.



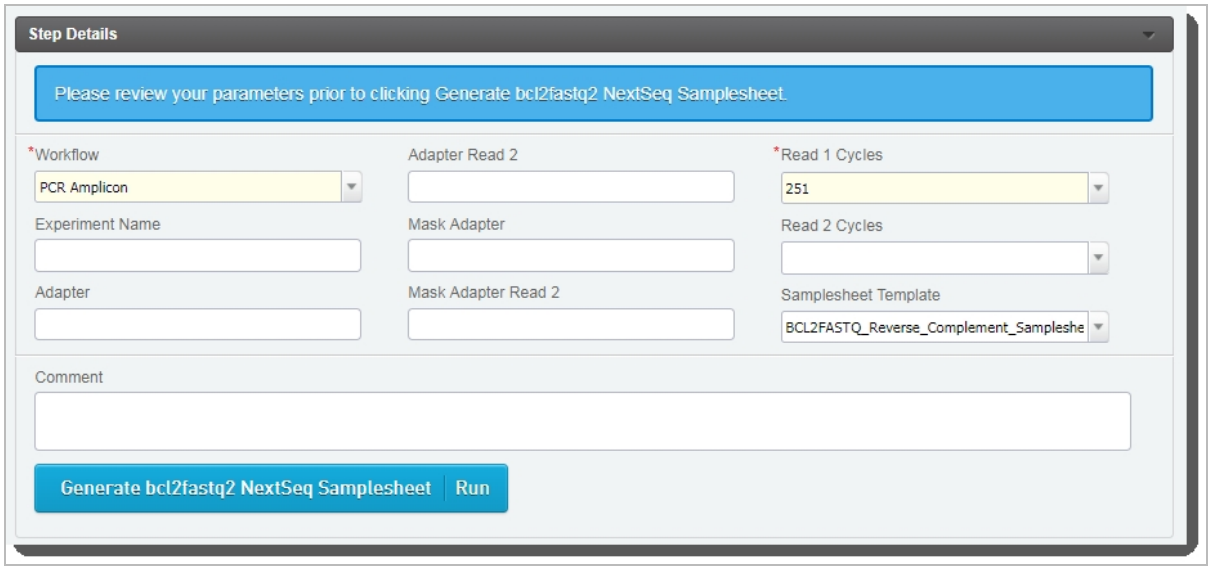
   c. Click Record Details.
5. On the Record Details screen:
   a. Under Reagent Lot Tracking, select the reagent lot used in the step.
   b. You may need to add/activate the lot on the Reagents and Controlsscreen.

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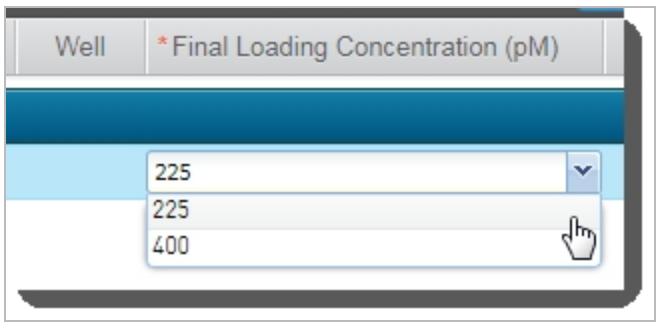


c. In the Step Details table, populate the fields as appropriate. Note the following:Workflow and Read 1 Cycles are required fields.

d. In the Samplesheet Template drop-down list, the reverse complement template is selected by default. Do not change this value.

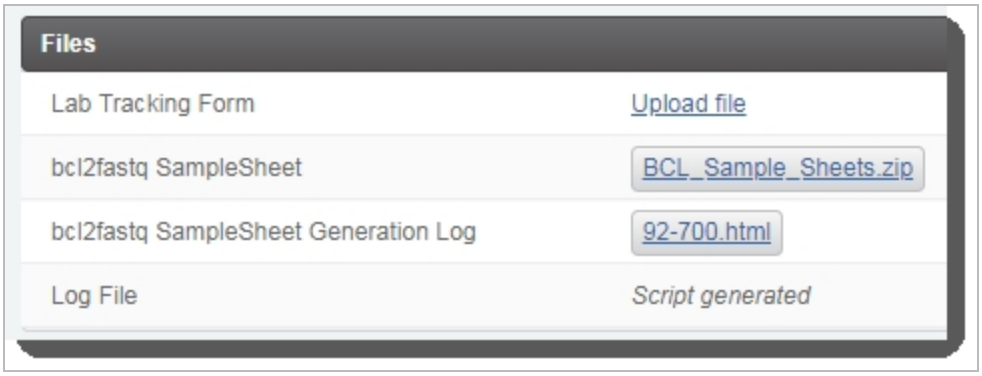


e. In the Sample Details table, enter the Final Loading Concentration. You may select from the two preset options - 225 (for PCR-free workflows) or400 (for Nano workflows), or enter a different value.



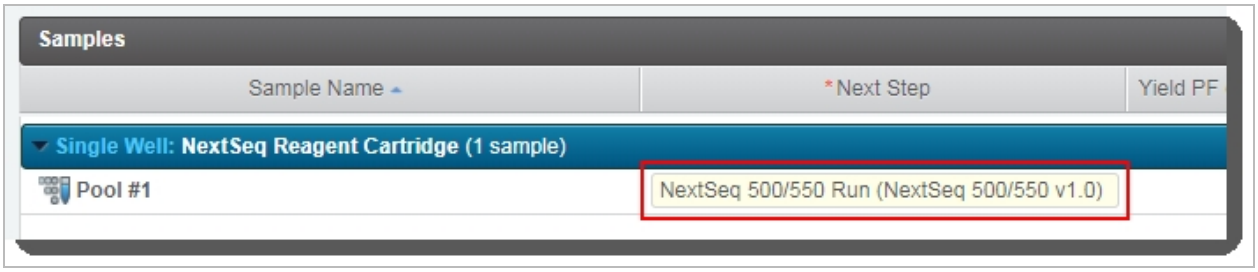
f. Click Generate bcl2fastq2 NextSeq SampleSheet.

g. The LIMS generates the samplesheet and attaches it and a log file to placeholders in the Files area of the Record Detailsscreen. Download the files and validate their format and content.



h. Click Next Steps.

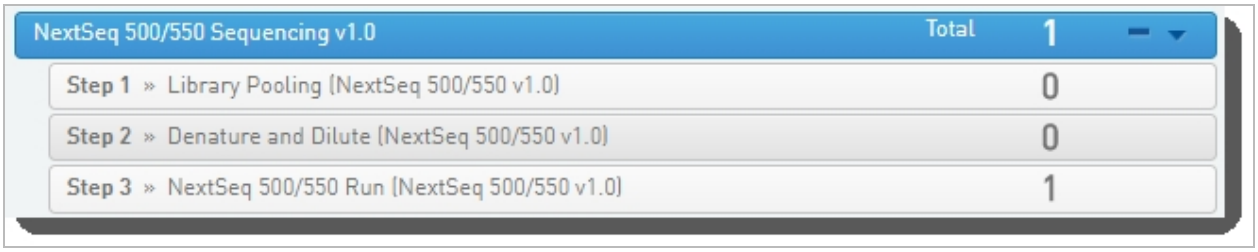
6. On the Assign Next Steps screen, samples are already assigned to the NextSeq 500/550 Run (NextSeq 500/550 v1.0) step.



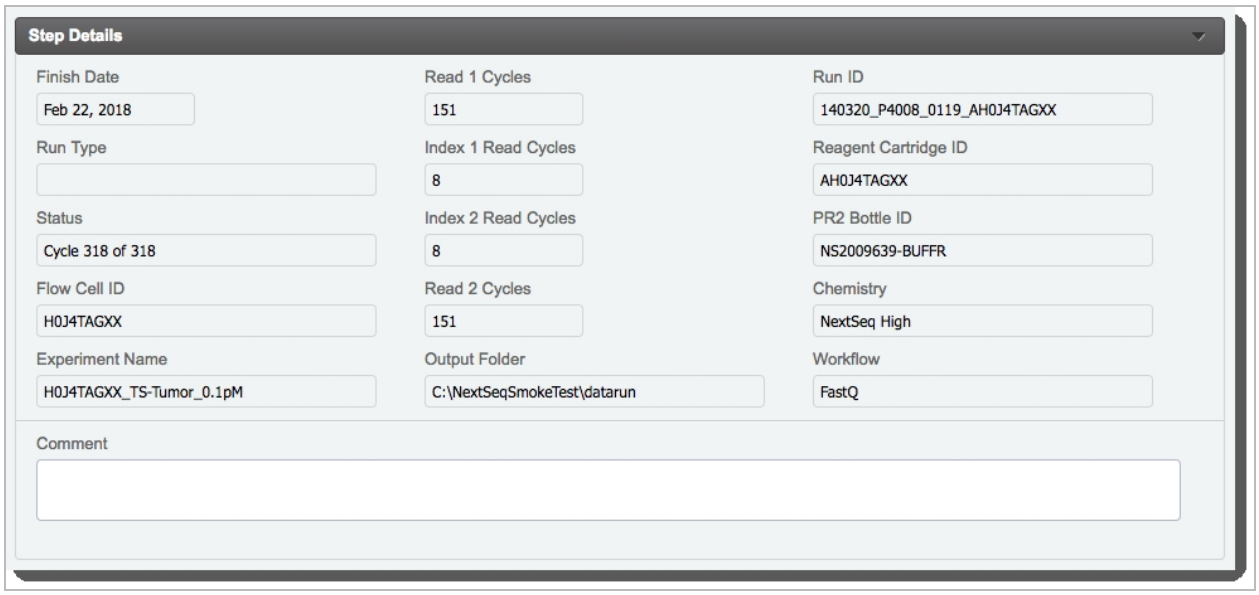
7. Click Finish Step.

3. Run NextSeq 500/550 Run (NextSeq 500/550 v1.0) Step

1. In Lab View, locate the NextSeq 500/550 Sequencing v1.0 protocol. You will see your pool of samples queued for the NextSeq 500/550 Run (NextSeq 500/550 v1.0) step. Click the step to proceed to the Queue screen.

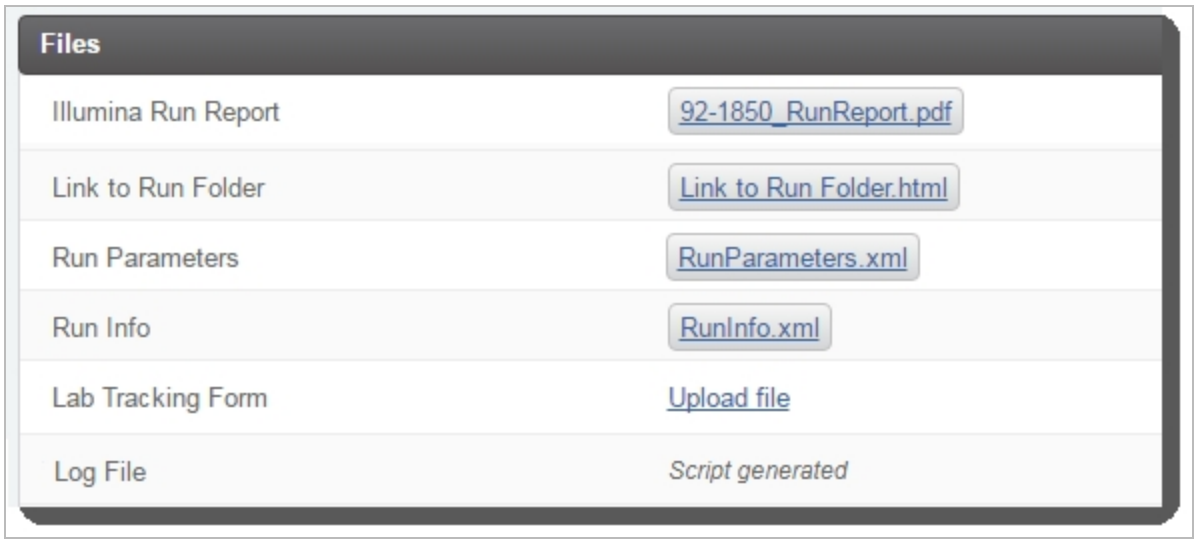


2. Add the pool to the Ice Bucket and click View Ice Bucket.
3. On the Ice Bucket screen, click Begin Work.
4. On the Record Details screen, the fields in the table are read-only. Once the run completes, the integration will automatically: Populate the fields.



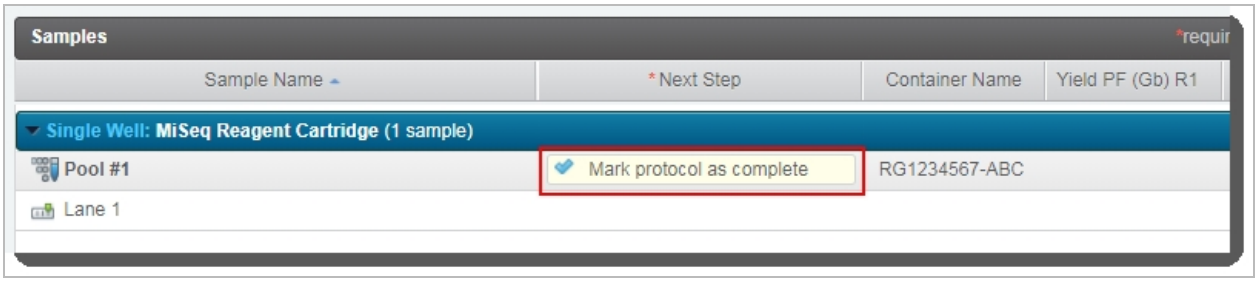
a. Attach files to the Illumina Run Report, Link to Run Folder, Run Parameters, and Run Info placeholders. (Note that the Log File is attached once the next step for samples has been assigned by the Next Step - Advanceautomation.)

b. Do not continue to step 5 and complete the step until the Illumina Run Report has been attached.



c. Populate the fields in the Sample Details table. See *User and Configuration Guide* for details.

5. Click Next Steps.
6. On the Assign Next Steps screen, the next step is already set to Mark protocol as complete.



7. Click Finish Step.

Validating Creation of Event Files

Follow the steps below to confirm that event files are created by the batch file in the destination path. The steps assume that the default configuration has been successfully imported, and support for NCS v4.0 has been configured.

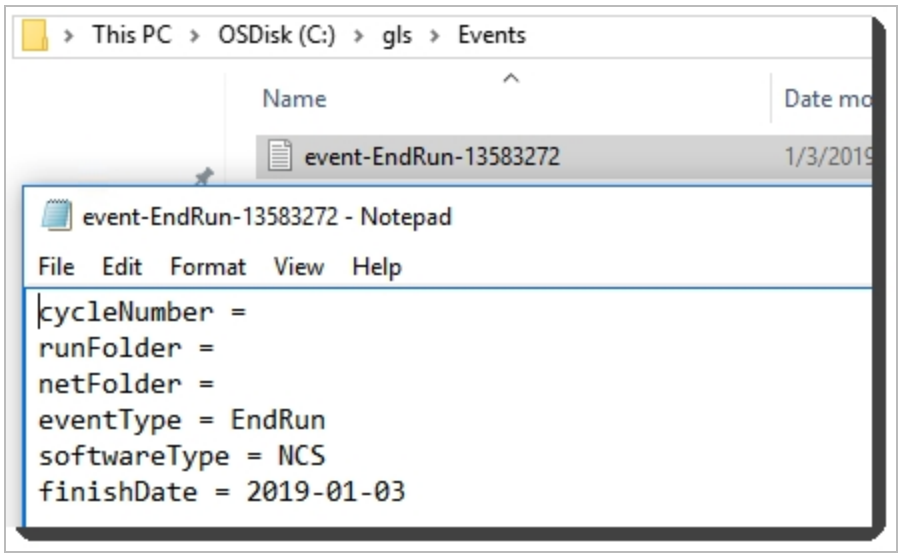
This test validates that:

- Your DESTINATION_PATH is correctly configured.
- The instrument computer can access and write to the DESTINATION_PATH.
- There are no syntax errors in the LIMS batch file.

You can validate the gls_event_ncs_rta.bat batch file by executing it manually:

1. Edit `gls_event_ncs_rta.bat` using any text editor (Notepad++ recommended). By default, this file is installed to `C:\gls`.
2. In the code line set `DESTINATION_PATH=C:\gls\Events\`, do the following:
   Change `C:\gls\Events\` to the network path of the event files directory. Remember to include the trailing "\" at the end of the file path.
3. Save your changes.
4. Manually executing the batch file will create a dummy EndRun event file in the directory that you defined in the previous step.

The content of the dummy file is shown below:



Troubleshooting

If an automation trigger does not appear to run its corresponding scripts, see:

- *Troubleshooting Automation Worker* in the *Administration* section of the *Clarity LIMS v6 Core Functionality* documentation.
- *Troubleshooting Automation* in the *Automation* section of the *Clarity LIMS API & Database* documentation.

If an error occurs that does not provide direction on how to proceed:

- Confirm the version of the installed Illumina NextSeq Integration Package by running the following command from the server console:

```
rpm -qa | grep -i nextseq
```

If the error is related to data parsing, retrieving run results data, or report values not appearing as expected:

- Review the `NextSeqIntegrator.log` file, located at `/opt/gls/clarity/extensions/Illumina_NextSeq/v2/SequencingService`

Additional troubleshooting information for this integration is provided on the *Illumina Instrument Integrations FAQ* on page 2598 page.

If you are unable to resolve the issue, contact the BaseSpace Clarity LIMS Support team, supplying the relevant information from the troubleshooting already performed.

Illumina NextSeq 500/550 Integration v2.0.3 Release Notes

Compatibility:

- BaseSpace Clarity LIMS v4.2 to v5.x
- NGS Extensions Package v5.17 or later
- Preconfigured Workflows Package (PCWP) v1.x

Illumina NextSeq Software Compatibility:

- NextSeq Control Software (NCS) v4.0

This integration only supports Standalone mode on NCS v4.0.

Features to note for this release

This release includes the following:

- Support for NextSeq Control Software (NCS) v4.0The NextSeq batch file (gls_event_ncs_rta.bat) updated to pull the sequencing run output folder network path from the RunParameter.xml file, as NCS v4.0 no longer outputs the path as a string.

Issues resolved in this release

The following issues were resolved in this release:

- Incomplete content in Runreport.pdf
- Incorrect FinishDate in EndRun event file

Documentation

For step-by-step instructions on how to validate the NextSeq integration with NCS v4.0, please refer to the Validating creation of event files section of the *Illumina NextSeq 500/550 Integration v2.0.3 - Validation and Troubleshooting Guide*,

Illumina NextSeq 500/550 Integration v2.0 Configuration and User Interaction

Contents

Introduction

The Illumina NextSeq 500/550 Integration Package v2.0 supports the integration of BaseSpace Clarity LIMS to Illumina NextSeq 500 and 550 sequencers.

The integration allows for automated tracking of an Illumina sequencing run in the LIMS. This includes tracking instrument run status, generating run report, and capturing and parsing run statistics. In addition, this integration provides automated generation of a sample sheet file for use with bcl2fastq2 v2.20.0 analysis software.

- This guide describes the integration between the LIMS and the Illumina NextSeq system. It includes information about protocols and automations, configuration options, installed components, and rules and constraints.
- For instructions on validating and troubleshooting the Illumina NextSeq 500/550 Integration Package, see the *Illumina NextSeq 500/550 Integration v2.0 Validation and Troubleshooting* on page 1934.
- For overview information about how the Illumina – LIMS interface works, and which events are monitored, see *How the Illumina Instrument and LIMS Software Interact*.

Overview

The Illumina NextSeq 500/550 Integration Package v2.0 includes:

- Preconfigured NextSeq 500/550 Sequencing v1.0 workflow that maps to lab protocol steps and instrument runs.
- Automated generation of a sample sheet for use with bcl2fastq2 v2.20.0 analysis software.Automated tracking of the NextSeq sequencing run and parsing of run statistics into the LIMS, including:Run status and metrics of sequencing run
  - Sequencing run parameters
  - Real Time Analysis v2 (RTA2) run directory location and other run specific information

Information on these features is provided in this guide.

Note that this integration also includes a preconfigured Library Prep Validation v1.0 workflow, used for validation purposes only. The workflow contains a single-step protocol that models the library prep required to produce normalized libraries that are ready for the NextSeq 500/550 Sequencing v1.0 workflow. For details, see the *Illumina NextSeq 500/550 Integration v2.0 Validation and Troubleshooting* on page 1934.

Prerequisites and Assumptions

It is assumed that samples enter the NextSeq 500/550 Sequencing v1.0 workflow as normalized libraries, and have had reagent labels attached. That is, before they are assigned to the workflow:

- Samples have been accessioned into the LIMS.
- Samples have been run through QC and library prep.
- Samples have been normalized, and the value is captured in a field called Normalized Molarity (nM).

For details on sample accessioning, see the following articles in the LIMS Documentation (Samples section):

- *Sample Accessioning*
- *Using Sample Lists to Upload and Modify Samples*

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Samples can be assigned to the NextSeq 500/550 Sequencing v1.0 workflow automatically (using a routing script), or manually from the Projects and Samples dashboard (see the *Assigning Samples to Workflows* article).

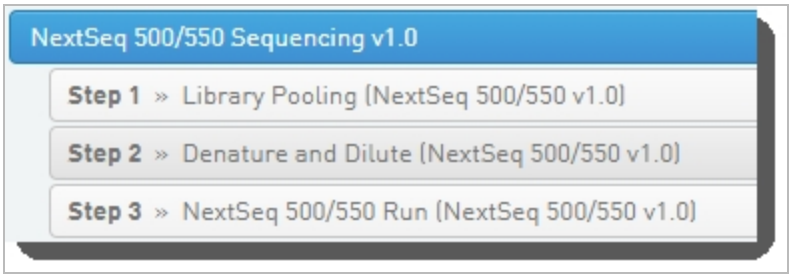
Workflows, Protocols, and Steps

The Illumina NextSeq Integration includes the NextSeq 500/550 Sequencing v1.0 workflow, which contains a single protocol of the same name.

The NextSeq 500/550 Sequencing v1.0 protocol includes the following steps:

- Library Pooling (NextSeq 500/550 v1.0)
- Denature and Dilute (NextSeq 500/550 v1.0)
- NextSeq 500/550 Run (NextSeq 500/550 v1.0)

The following image shows the protocol and steps as displayed to the lab scientist working in BaseSpace Clarity LIMS Lab View.



Step Automations

Table 1 lists the step automations configured on the NextSeq 500/550 Sequencing v1.0 protocol steps. Automation details are provided in the Configuration details sections following the table.

Figure 3 Table 1: NextSeq 500/550 Sequencing v1.0 protocol step automations

Step	Automation
1. Library Pooling (NextSeq 500/550 v1.0)	• Set Next Step - Advance
2. Denature and Dilute (NextSeq 500/550 v1.0)	• Generate bcl2fastq2 NextSeq 500/550 SampleSheet • Set Next Step - Advance
3. NextSeq 500/550 Run (NextSeq 500/550 v1.0)	• Set Next Step - Advance

Configuration Details

The sections below describe the configuration installed with the Illumina NextSeq Integration.

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The Illumina NextSeq 500/550 Integration Package v2.0 supports BaseSpace Clarity LIMS v4.2.x and v5.x.

As of LIMS v5, the term user-defined field (UDF) has been replaced with custom field in the user interface (the API resource is still called udf). There are two types of custom fields:

- Master step fields - configured on master steps. These fields only apply to the master step on which they are configured, and the steps derived from those master steps.
- Global fields - configured on entities such as submitted sample, derived sample, measurement, etc. These fields apply to the whole LIMS system.

Step 1: Library Pooling (NextSeq 500/550 v1.0)

Note that in LIMS v5, the Library Pooling (NextSeq 500/550 v1.0) step is derived from the Library Pooling v1.0 master step.

In this step, user manually places libraries into pools.

Automations

One automation is configured on this step: Set Next Step - Advance

Figure 4 Automation configuration on Library Pooling (NextSeq 500/550 v1.0) step - LIMS v4.2

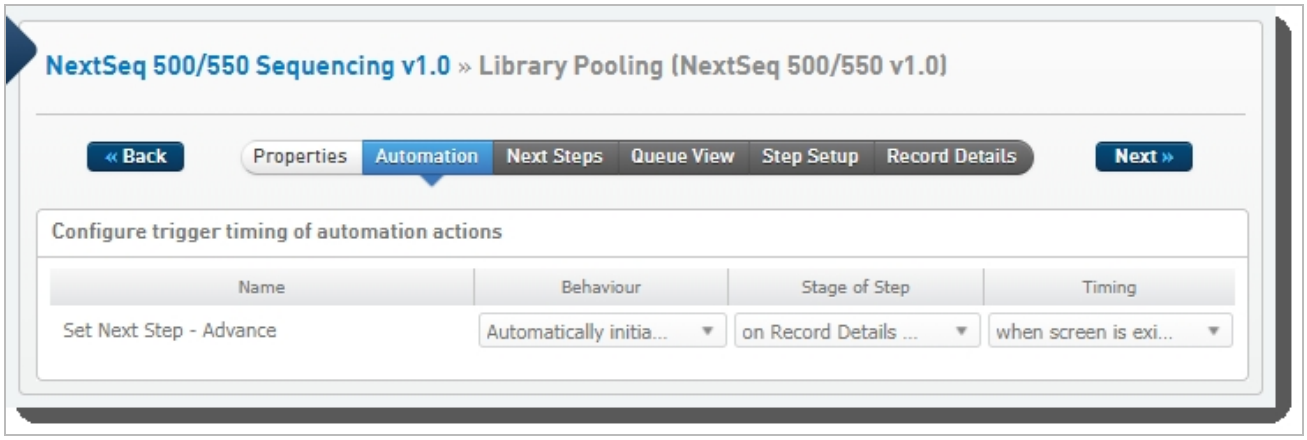
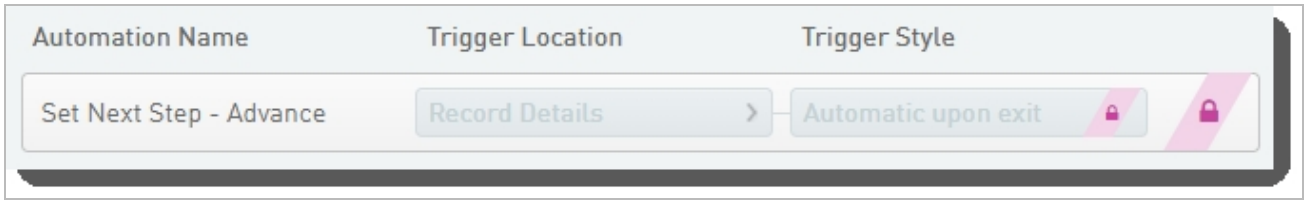


Figure 5 Automation configuration on Library Pooling (NextSeq 500/550 v1.0) step - LIMS v5 and later



Set Next Step - Advance Automation

Automatically triggered on exit of the Record Details screen, this automation advances samples to the next step in the protocol.

Default command line:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \ -t false \ -h false \ -exp 'nextStep =
::ADVANCE::' \ -log {compoundOutputFileLuid0}"
```

Step UDFs/Custom Fields

There is one field configured on the Library Pooling (NextSeq 500/550 v1.0) step. This field displays on the Record Details screen at run time.

The field is shown below in the BaseSpace Clarity LIMS v5 Configuration area (Custom Fields > Master Step Fields tab). See Table 2 for LIMS v4.2 and LIMS v5 field configuration details.

Figure 6 Table 2: Library Pooling (NextSeq 500/550 v1.0) step UDF/custom field configuration



Field Name	Field Type	Field Constraints/Options
Comment	Multi-line Text	• Required: No • Read Only: No

Global UDFs/Custom Fields

Table 3 lists the global UDFs/fields that are configured to display on the Queue and Ice Bucket screens of the Library Pooling (NextSeq 500/550 v1.0) step. Note that the majority of these fields display in expanded view only.

Figure 7 Table 3: Global UDF/custom field configuration for Library Pooling (NextSeq 500/550 v1.0)

Field Name	Field Name	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Configured on Sample in LIMS v4.2 and Submitted Samplein LIMS v5:			

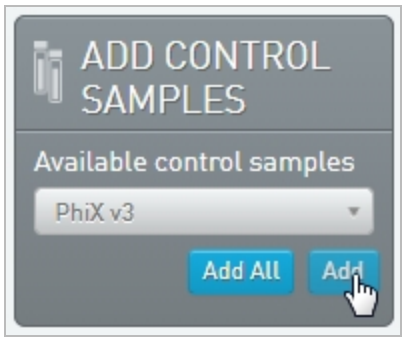
For Research Use Only. Not for use in diagnostic procedures.

Field Name	Field Name	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Application	Single-line text (v4.2) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: Yes	• TruSeq mRNA sequencing • TruSeq DNA sequencing (large genome de novo) • TruSeq DNA sequencing (large genome re-seq) • TruSeq DNA sequencing (small genome de novo) • TruSeq DNA sequencing (small genome re-seq) • Nextera DNA sequencing • TruSeq Custom Amplicon sequencing • ChIP-sequencing • Exome sequencing • Mate pair sequencing • Small RNA Sequencing
Pooling	Single-line text (v4.2) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: Yes	• Yes • No
Read Length	Single-line text (v4.2) Text (v5)	• Required: No • Read Only: No	
Sequencing Coverage	Single-line text (v4.2) Text (v5)	• Required: No • Read Only: No	
Sequencing Method	Single-line text (v4.2) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: Yes	• Single Read • Paired End Read • Indexed Single read • Indexed Paired End Read
Configured on Analyte in LIMS v4.2 and Derived Samplein LIMS v5:			
Normalized Molarity (nM)	Numeric	• Required: No • Read Only: No	• Decimal Places Displayed: 2

User Interaction

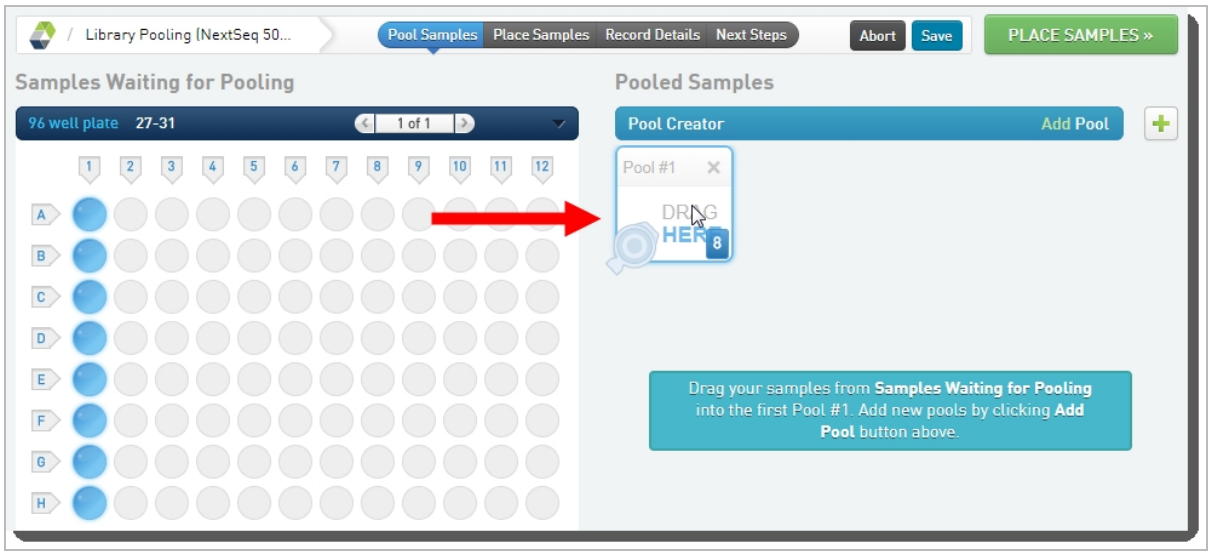
1. On the Queue screen:
   a. Add the samples to the Ice Bucket.
   b. In the Add Control Samples panel, add the PhiX v3 control sample to the Ice Bucket.

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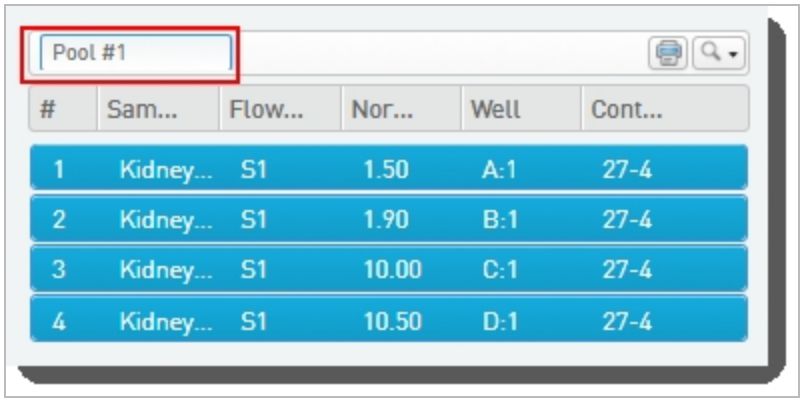


c. Click View Ice Bucket.

2. On the Ice Bucket screen, click Begin Work.
3. On the Pool Samples screen:
   a. Create a pool of samples by dragging samples into the Pool Creator.



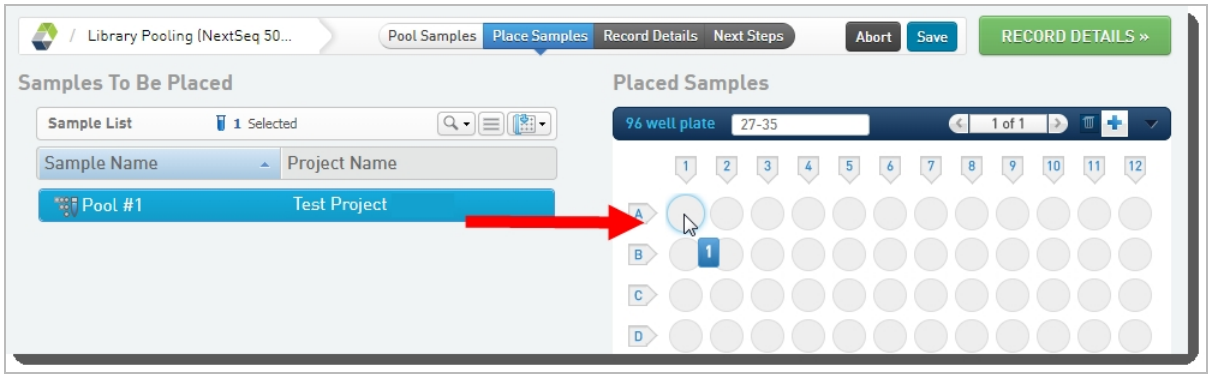
   b. Name the pool, or accept the default name - Pool #1.



   c. Click Place Samples.

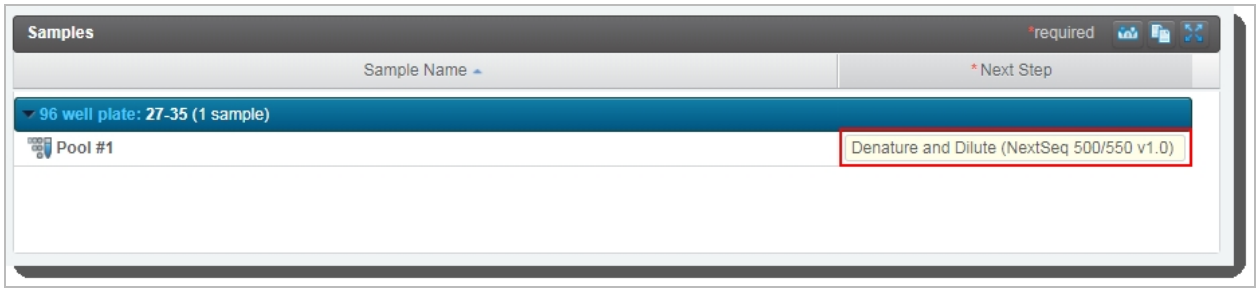
4. On the Placement screen:

a. Select the pool in the Samples to be Placed area on the left and drag it over to the container on the right.



b. Click Record Details.

5. On the Record Details screen, click Next Steps.
   On the Assign Next Steps screen, next step is already set to Denature and Dilute (NextSeq 500/500 v1.0).



6. Click Finish Step.

Step 2: Denature and Dilute (NextSeq 500/550 v1.0)

In this step, pooled libraries are denatured and diluted and placed into the reagent cartridge that will be loaded into the NextSeq instrument.

Automations

The following automations are configured on this step:

- Generate bclfastq2 NextSeq SampleSheet
- Set Next Step - Advance

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Figure 8 Automation configuration on Denature and Dilute (NextSeq 500/550 v1.0) step - LIMS v4.2

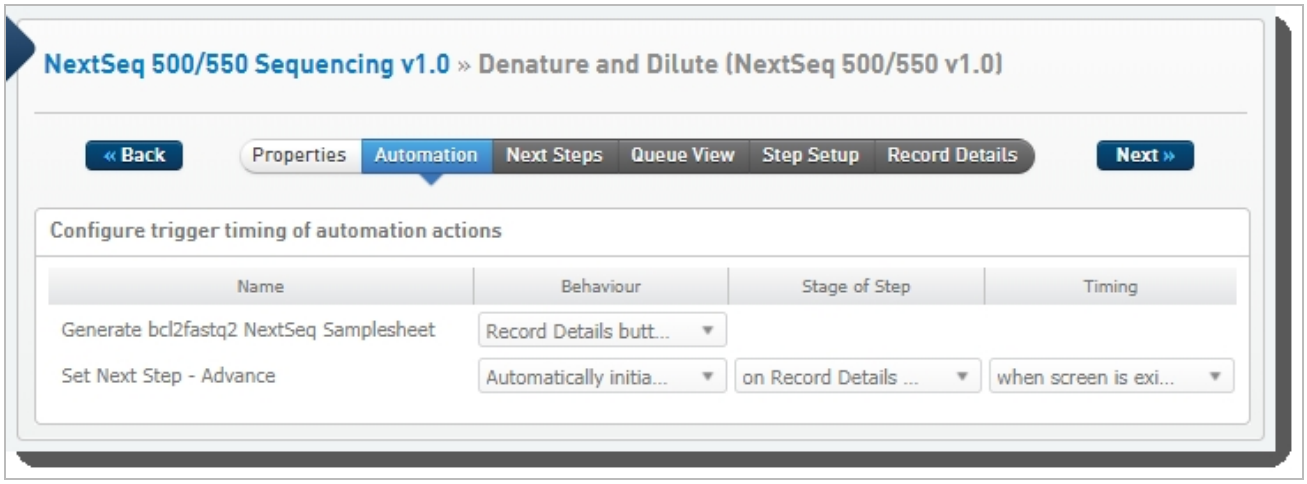
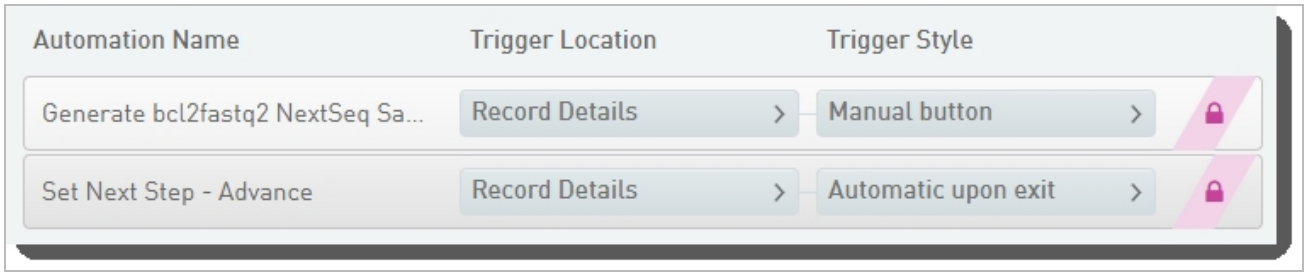


Figure 9 Automation configuration on Denature and Dilute (NextSeq 500/550 v1.0) step - LIMS v5 and later



Generate bcl2fastq2 NextSeq SampleSheet Automation

Triggered when the user clicks a button on the Record Details screen, this automation generates generates the sample sheet and attaches it to the step. For details, see Sample sheet generation section, below.

Default command line:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/DriverFileGenerator.jar -i {processURI:v2} -u {username} -p {password} \ script:driver_file_generator \ -t /opt/gls/clarity/extensions/conf/driverfiletemplates/{udf:Samplesheet Template} \ -o {compoundOutputFileLuid1}.csv \ -q true \ -destLIMSID {compoundOutputFileLuid1} \ -l {compoundOutputFileLuid2}"
```

Set Next Step - Advance Automation

Automatically triggered on exit of the Record Details screen, this automation advances samples to the next step in the protocol.

For Research Use Only. Not for use in diagnostic procedures.

In LIMS v4.2, the automation also invokes the setUDF script, which sets the value of the Progress UDF. The LIMS updates this UDF value as samples progress through the workflow steps. The Progress UDF does not display in the LIMS user interface, but it does display in the LabLink Collaborations Interface so that collaborators can see the status of their samples.

Default command line (LIMS v4.2):

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -u {username} -p {password} \ script:setUDF \ -i {processURI:v2} \ -f 'Progress' \ -t '//input/@uri->//sample/@uri' \ -v 'Library ready for sequencing' \ script:evaluateDynamicExpression \ -i {stepURI:v2:http} \ -t false \ -h false \ -exp 'nextStep = ::ADVANCE::' \ -log {compoundOutputFileLuid3}"
```

Default command line (LIMS v5):

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression \ -t false \ -h false \ -exp 'nextStep = ::ADVANCE::' \ -log {compoundOutputFileLuid3}"
```

Step UDFs/Custom Fields

There are 10 fields configured on the Denature and Dilute (NextSeq 500/550 v1.0) step. The fields are shown below in the BaseSpace Clarity LIMS v5 Configuration area (Custom Fields > Master Step Fields tab).

At run time, these fields display on the Record Details screen, in the Step Data table. The user manually populates the fields (see User interaction) and the values are then used to generate the sample sheet. For details on the sample generation functionality, see Sample sheet generation section below.

See Table 4 for LIMS v4.2 and LIMS v5 field configuration details.

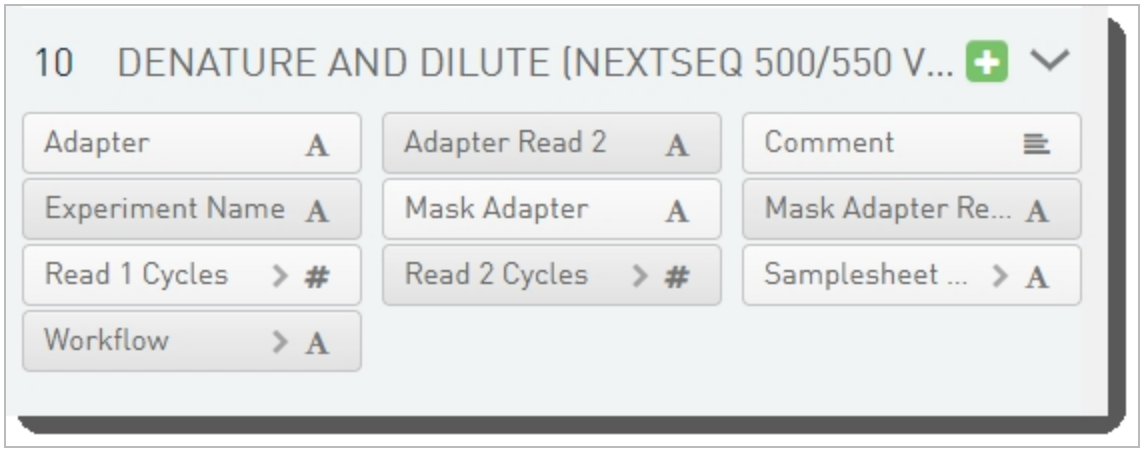


Figure 10 Table 4: Denature and Dilute (NextSeq 500/550 v1.0) step UDF/custom field configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Adapter	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: No	
Adapter Read 2	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: No	
Experiment Name	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: No	
Mask Adapter	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: No	
Mask Adapter Read 2	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: No	
Read 1 Cycles	Numeric (v4.2) Numeric Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: Yes	• 251 (Default) • 151 • 101 • 76 • 51 • Range From: 0 to 1000 • Decimal Places Displayed: 0
Read 2 Cycles	Numeric (v4.2) Numeric Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: Yes	• 251 • 151 • 101 • 76 • 51 • Range From: 0 to 1000 • Decimal Places Displayed: 0
SampleSheet Template	Single-line Text (v4.2) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: No	• BCL2FASTQ_Reverse_Complement_Samplesheet.csv (default) • BCL2FASTQ_Samplesheet.csv

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Workflow	Single-line Text (v4.2) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: No	• Assembly • Custom Amplicon • Enrichment • GenerateFASTQ • LibraryQC • Metagenomics • PCR Amplicon • Resequencing

Groups of Defaults/Presets

The following group of defaults/presets are configured for Denature and Dilute (NextSeq 500/550 v1.0) step UDFs/master step custom fields:

Resequencing

- Read 1 Cycles: 251
- Workflow: Resequencing

Custom Amplicon

- Read 1 Cycles: 251
- Workflow: Custom Amplicon

PCR Amplicon

- Read 1 Cycles: 251
- Workflow: PCR Amplicon

Enrichment

- Read 1 Cycles: 251
- Workflow: Enrichment

Library QC

- Read 1 Cycles: 251
- Workflow: LibraryQC

Metagenomics

- Read 1 Cycles: 251
- Workflow: Metagenomics

GenerateFastQ

- Read 1 Cycles: 251
- Workflow: GenerateFastQ

Assembly

- Read 1 Cycles: 251
- Workflow: Assembly

Global UDFs/Custom Fields

Table 5 shows the global UDFs/fields that are configured to display on the Queue, Ice Bucket and Record Details screens of the Denature and Dilute (NextSeq 500/550 v1.0) step.

Figure 11 Table 5: Global UDF/custom field configuration for Denature and Dilute (NextSeq 500/550 v1.0) step

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Configured on Sample in LIMS v4.2 and Submitted Samplein LIMS v5:			
Progress *(LIMS v4.2) Displays in LabLink Collaborations Interface only*	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: No • Custom Entries: Yes	
Read Length *Displays on Queue and Ice Bucket screens*	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: No	
Sequencing Method *Displays on Queue and Ice Bucket screens*	Single-line Text (v4.2) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: Yes	• Single Read • Paired End Read • Indexed Single Read • Indexed Paired End Read
Configured on Analyte in LIMS v4.2 and Derived Samplein LIMS v5:			
Final Loading Concentration *Displays on Record Details screen*	Numeric (v4.2) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 225 • 400

Step File Placeholders

Placeholders for the following files are configured on the Record Details screen of the Denature and Dilute (NextSeq 500/550 v1.0) step:

- Lab Tracking Form (manually generated)
- bcl2fastq2 Samplesheet (auto-generated)

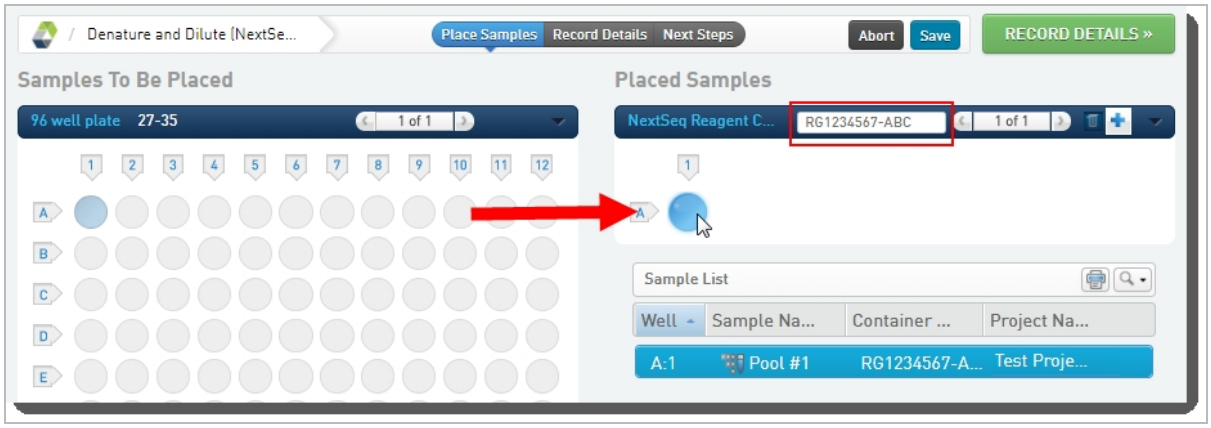
- bcl2fastq2 Samplesheet Generation Log (auto-generated)
- Log File (auto-generated)

Figure 12 Table 6: Sample sheet files and placeholders generated by Denature and Dilute (NextSeq 500/550 v1.0) step

Item	Description
Lab Tracking Form placeholder	This placeholder in the LIMS allows the user to manually attach a lab-specific tracking form to the step.
bcl2fastq2 SampleSheet	This CSV file is automatically generated by the LIMS for use with the bcl2fastq2 v2.20.0 analysis software. May be opened as a text file, or as an MS Excel spreadsheet.
bcl2fastq2 SampleSheet Generation Log	Automatically generated by the LIMS, this log file captures any errors that the LIMS may encounter when generating the sample sheet.
Log File	Automatically generated by the LIMS, this log file captures the status of the EvaluateDynamicExpression script that is invoked by the Set Next Step - Advanceautomation.

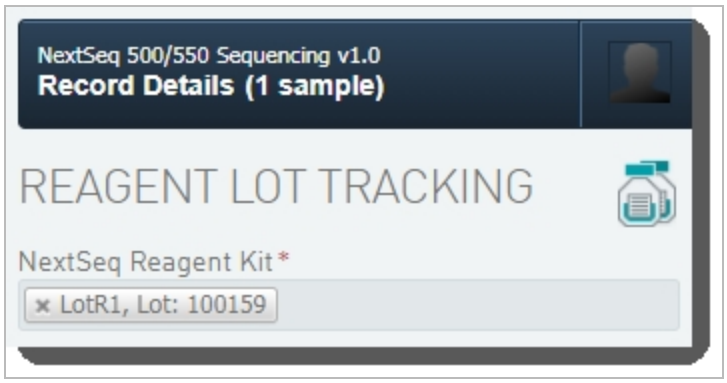
User Interaction

1. On the Queue screen, add the pool to the Ice Bucket and click View Ice Bucket.
2. On the Ice Bucket screen, click Begin Work.
3. On the Placement screen:
   a. Scan the barcode of the NextSeq reagent cartridge into the NextSeq Reagent Cartridge field.
   b. Place the pool of samples into the reagent cartridge.

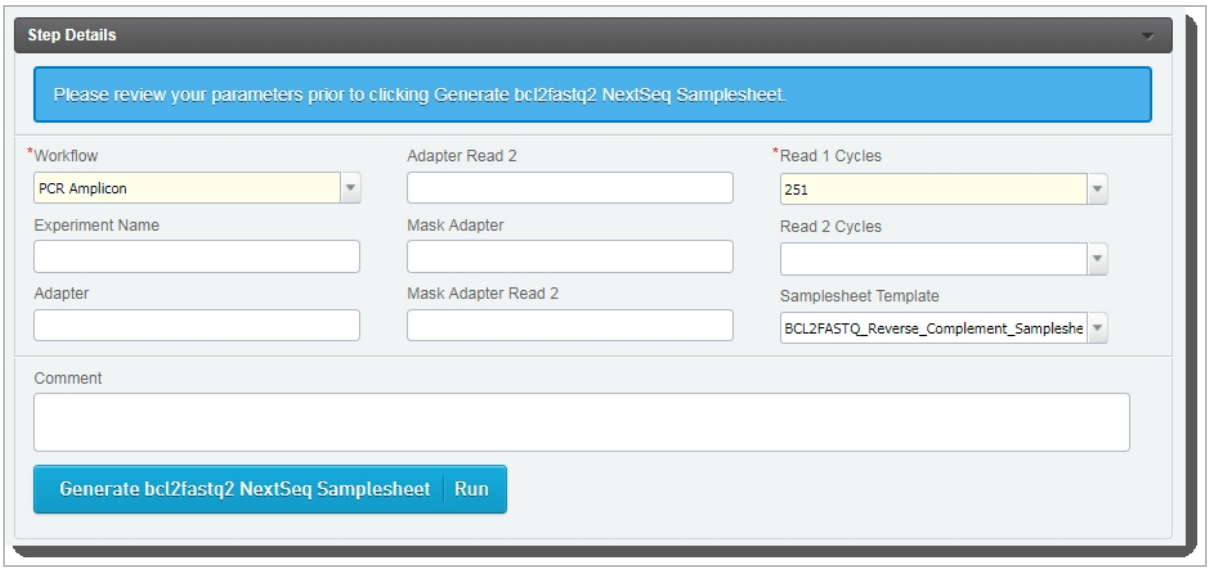


   c. Click Record Details.
4. On the Record Details screen:
   a. Under Reagent Lot Tracking, select the reagent lot used in the step (you may need to add/activate the lot on the Reagents and Controlsscreen).

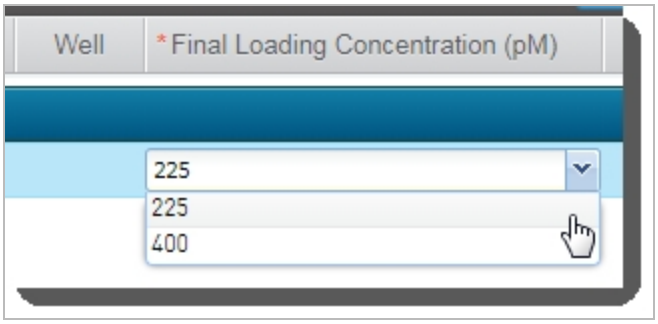
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b. Populate the fields in the Step Details table, as appropriate. Note that Workflow and Read 1 Cycles are required fields.

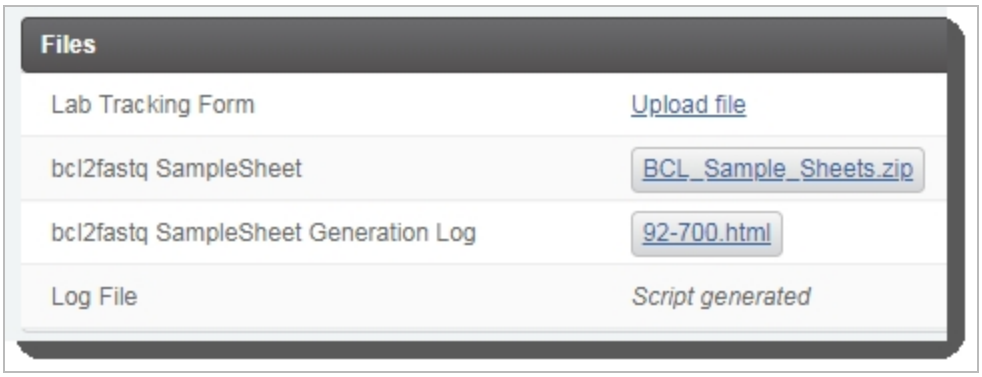


c. In the Sample Details table, enter the Final Loading Concentration. You may select from the two preset options - 225 (for PCR-free workflows) or400 (for Nano workflows), or enter a different value.



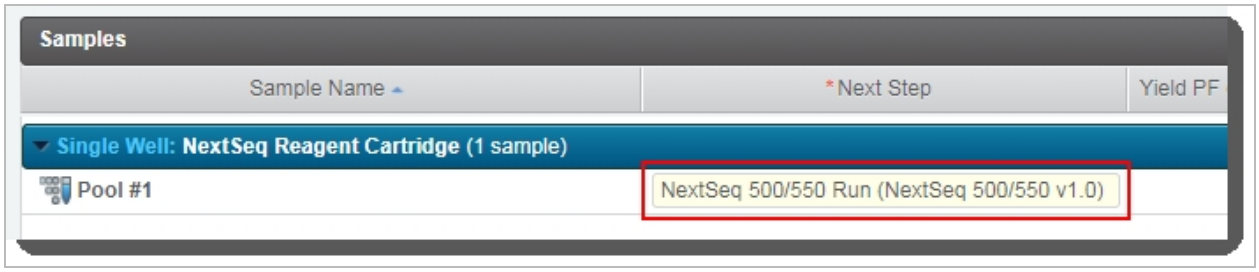
d. Click Generate bcl2fastq2 NextSeq SampleSheet.

e. The LIMS generates the samplesheet and attaches it and a log file to placeholders in the Files area of the Record Detailsscreen. Download the files and validate their format and content.



f. Click Next Steps.

5. On the Assign Next Steps screen, samples are already assigned to the NextSeq 500/550 Run (NextSeq 500/550 v1.0) step.



6. Click Finish Step.

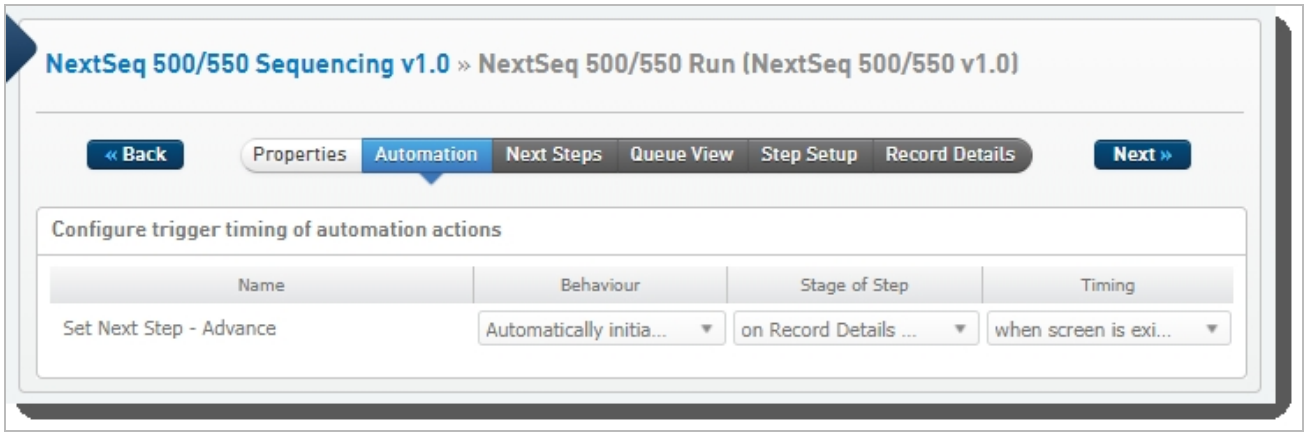
See also Sample sheet generation section.

Step 3: NextSeq 500/550 Run (NextSeq 500/550 v1.0)

Automations

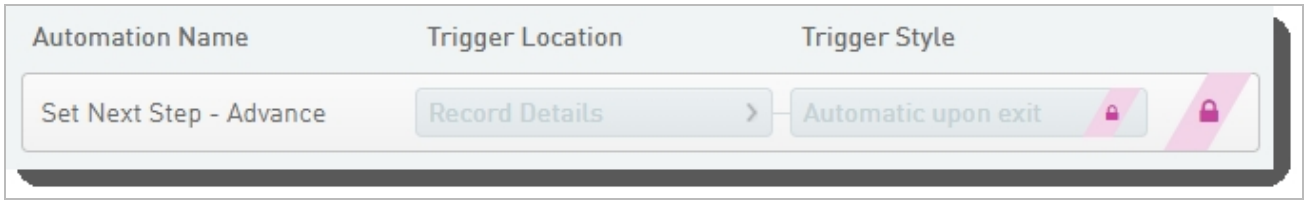
One automation is configured on this step: Set Next Step - Advance

Figure 13 Automation configuration on NextSeq 500/550 Run (NextSeq 500/550 v1.0) step - LIMS v4.2



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Figure 14 Automation configuration on NextSeq 500/550 Run (NextSeq 500/550 v1.0) step - LIMS v5 and later



Set Next Step - Advance Automation

Automatically triggered on exit of the Record Details screen, this automation advances samples to the next step in the protocol.

Default command line:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression \ -t false \ -h false \ -exp 'nextStep = ::ADVANCE::' \ -log {compoundOutputFileLuid5}"
```

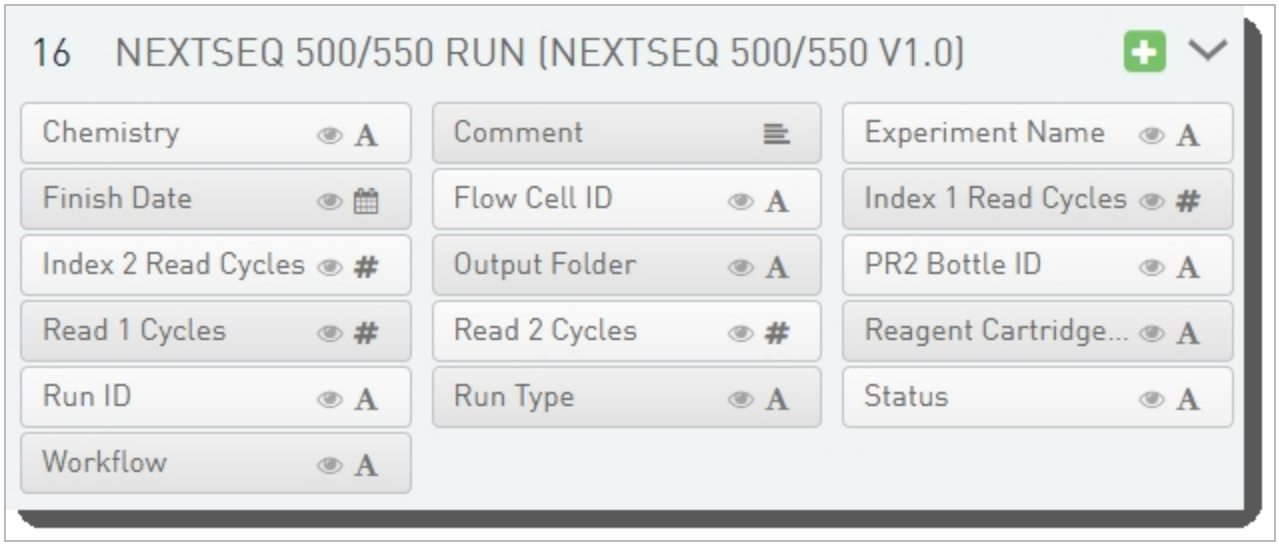
Step UDFs/Custom Fields

There are 16 fields configured on the NextSeq 500/550 Run (NextSeq 500/550 v1.0) step. These fields display on the Record Details screen at run time.

Some of the field values are completed by the user running the step, the remaining fields are automatically populated at the end of the run.

The fields are shown below in the BaseSpace Clarity LIMS v5 Configuration area (Custom Fields > Master Step Fields tab).

See Table 7 for LIMS v4.2 and LIMS v5 field configuration details.



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Figure 15 Table 7: NextSeq 500/550 Run (NextSeq 500/550 v1.0) step UDF / custom field configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Chemistry	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: Yes	
Comment	Multi-line Text	• Required: No • Read Only: No	
Experiment Name	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: Yes	
Finish Date	Date	• Required: No • Read Only: Yes	
Flow Cell ID	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: Yes	
Index 1 Read Cycles	Numeric	• Required: No • Read Only: Yes	Decimal Places Displayed: 0
Index 2 Read Cycles	Numeric	• Required: No • Read Only: Yes	Decimal Places Displayed: 0
Output Folder	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: Yes	
PR2 Bottle ID	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: Yes	
Read 1 Cycles	Numeric	• Required: No • Read Only: Yes	Decimal Places Displayed: 0
Read 2 Cycles	Numeric	• Required: No • Read Only: Yes	Decimal Places Displayed: 0
Reagent Cartridge ID	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: Yes	
Run ID	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: Yes	

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Dropdown Items
Run Type	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: Yes	
Status	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: Yes	
Workflow	Single-line Text (v4.2) Text (v5)	• Required: No • Read Only: Yes	

Global UDFs/Custom Fields

There are a number of sample and measurement global UDFs/fields that are configured to display on the Record Details screen of the NextSeq 500/550 Run (NextSeq 500/550 v1.0) step. These fields are auto-populated at the end of the sequencing run.

For more information, see the User interaction and Sequencing results parsing sections below.

Step File Placeholders

Placeholders for the following files are configured on the Record Details screen of the NextSeq 500/550 Run (NextSeq 500/550 v1.0) step:

- Illumina Run Report (auto-generated)
- Link to Run Folder (auto-generated)
- Run Parameters (auto-generated)
- Run Info (auto-generated)
- Lab Tracking Form (manually generated)
- Log File (auto-generated)

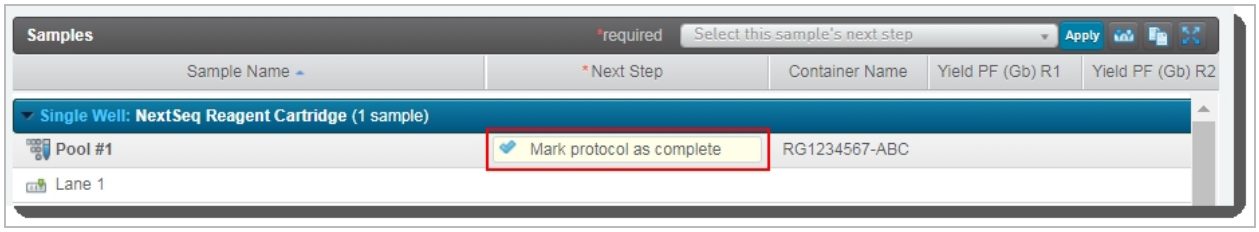
For details, see Generated and captured files section below.

User Interaction

1. On the Queue screen, add the pool to the Ice Bucket and click View Ice Bucket.
2. On the Ice Bucket screen, click Begin Work.
3. On the Record Details screen, the fields in the table are read-only. Once the run completes, the integration will automatically:
    - Populate the fields.
    - Attach files to the Illumina Run Report, Link to Run Folder, Run Parameters, and Run Info placeholders.
    - Populate the fields in the Sample Details table.

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4. Click Next Steps.
5. On the Assign Next Steps screen, the next step is already set to Mark protocol as complete.



6. Click Finish Step.

Sample Sheet Generation

Sample sheet generation occurs on the step prior to the sequencing run – Denature and Dilute (NextSeq 500/550 v1.0), which is the step where samples are placed on the container that will be loaded in the instrument.

The out-of-the-box configuration provides one automation, Generate bcl2fastq2 NextSeq SampleSheet, which uses the Template File Generator (DriverFileGenerator.jar) and a template file to generate a CSV format file for use with bcl2fastq2 v2.20.0 analysis software.

The sample sheet content is determined by the fields that display on the Record Details screen of the step (in the Step Data table), and the values that the user enters into these fields.

You can customize the template used to create the sample sheet, inserting adding additional columns to meet the needs of your lab.

For details:

- See the *Illumina Instrument Sample Sheets* article, which provides a sample template that you can download and customize for your lab.
- For instructions on customizing the template, see the *Creating Template Files* article.

Sequencing Results Parsing

The NextSeq 500/550 Run (NextSeq 500/550 v1.0) step records information for the flow cell lanes and generates a report summarizing the results. In addition, run parameters, run info, and a link to the run folder are automatically captured.

Generated and Captured Files

Table 8 describes the run information files, reports, placeholders, and links that the LIMS automatically generates or captures during a sequencing run.

Figure 16 Table 8: Run information generated or captured by NextSeq 500/550 Run (NextSeq 500/550 v1.0) step

Item	Description
Run Info *Run Parameters*	These XML files are automatically captured by the LIMS from the instrument's run folder. They include the key run parameters, many of which are parsed out into key step UDFs.
Link to Run Folder	Automatically generated by the LIMS, this is a link to the network run folder where the data that was captured from the instrument during the run is stored.
Illumina Run Report	Automatically generated by the LIMS, this report provides key information about the run and the samples on the flow cell. Information includes the flow cell ID, run directory location, and primary analysis metrics for the instrument – summarized per flow cell lane for the entire run, and individual reads in the case of paired-end runs. These metrics are compared against the instrument's per lane averages, calculated using metrics from the last 5 sequencing runs. Any values outside of 1 standard deviation are highlighted.
Lab Tracking Form placeholder	This placeholder in the LIMS allows the user to manually attach a lab-specific tracking form to the step.

Metadata

Listed below is the metadata that the LIMS automatically captures from the Illumina sequencing software as part of a sequencing run. This information is gathered from various run result files and events.

To see how these fields are configured in the LIMS, see Table 7.

- Chemistry
- Experiment Name – entered in software
- Finish Date* – run completion date
- Flow Cell ID
- Index 1 Read Cycles – intended Index cycles
- Index 2 Read Cycles – intended Index cycles
- Output Folder – run folder root
- PR2 Bottle ID
- Reagent Cartridge ID
- Read 1 Cycles
- Read 2 Cycles
- Run ID – the unique run ID

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- Run Type
- Status – current status of the sequencing run on the instrument
- Workflow

Finish Date is populated as follows:

- If the End Run event contains a date in the format YYYY-MM-DD, Finish Date will be set to the date in the event file.
- If the End Run event does not contain a date or the date is in the wrong format, Finish Date will be set to the date when the event file is processed.

Primary Analysis Metrics

Table 9 lists the Real Time Analysis v2 (RTA2) primary analysis metrics the LIMS automatically captures and records, per read, for samples in each flow cell lane. These metrics are captured upon run completion and are stored as UDFs/custom fields in the Record Details screen's Sample Details table.

Users can see both per read and per lane metrics by expanding the output.

Figure 17 Table 9: RTA primary analysis metrics captured by NextSeq 500/550 Run (NextSeq 500/550 v1.0) step

Per Read LIMS Field Name *(stored on derived sample/analyte input to the step)*	Per Lane LIMS Field Name *(stored in measurement placeholders in Record Details screen's Sample Details table)*
% Aligned R1	% Aligned R1
% Aligned R2	% Aligned R2
% Bases >=Q30 R1	% Bases >=Q30 R1
% Bases >=Q30 R2	% Bases >=Q30 R2
% Error Rate R1	% Error Rate R1
% Error Rate R2	% Error Rate R2
	% Phasing R1
	% Phasing R2
	% Prephasing R1
	% Prephasing R2
	%PF R1
	%PF R2
	Cluster Density (K/mm^2) R1
	Cluster Density (K/mm^2) R2
Intensity Cycle 1 R1	Intensity Cycle 1 R1
Intensity Cycle 1 R2	Intensity Cycle 1 R2

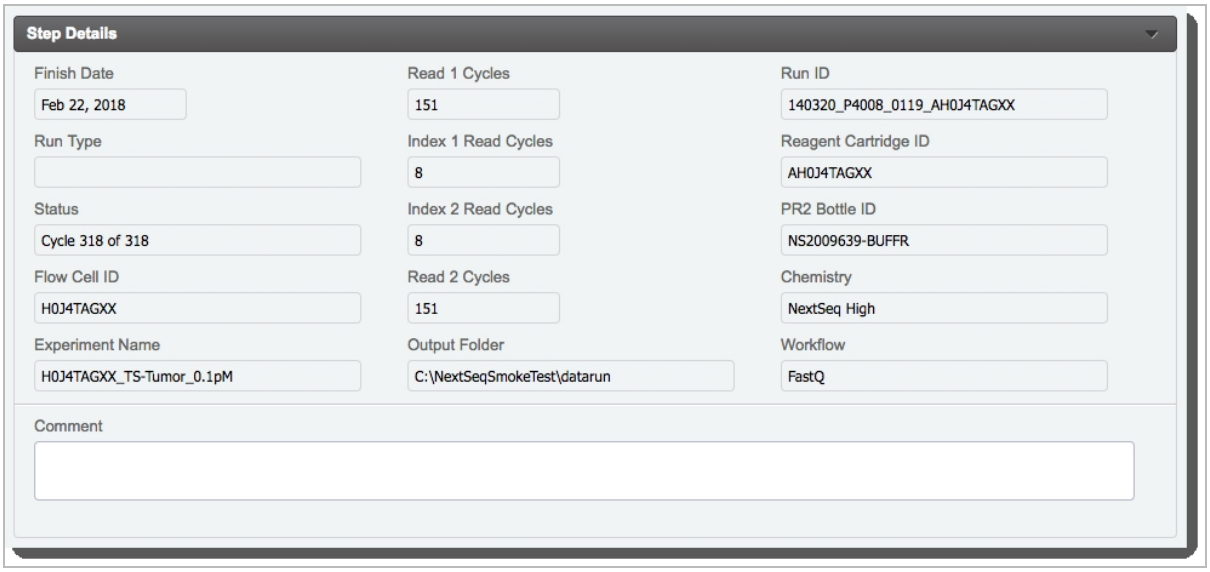
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Per Read LIMS Field Name *(stored on derived sample/analyte input to the step)*	Per Lane LIMS Field Name *(stored in measurement placeholders in Record Details screen's Sample Details table)*
	Reads PF (M) R1
	Reads PF (M) R2
Yield PF (Gb) R1	Yield PF (Gb) R1
Yield PF (Gb) R2	Yield PF (Gb) R2

How it Works

1. The sequencing service runs on the LIMS server. The service detects event files that the instrument software (RTA2) produces as the run progresses; this in turn lets the service know where to find the run data.
2. As the run data is written out and the End Run event is detected:
   a. The data is matched to the step based on the reagent cartridge ID, which was entered/scanned in by the user on the Denature and Dilute (NextSeq 500/550 v1.0) step.
   b. Read-only field values on the Record Details screen are populated accordingly.

   Figure 18 Record Details screen of NextSeq 500/550 Run (NextSeq 500/550 v1.0) step showing fields that are populated at the end of the instrument run



3. When it has finished processing the end run event and updating the fields in the LIMS, the sequencing service generates the report and attaches it to the step.

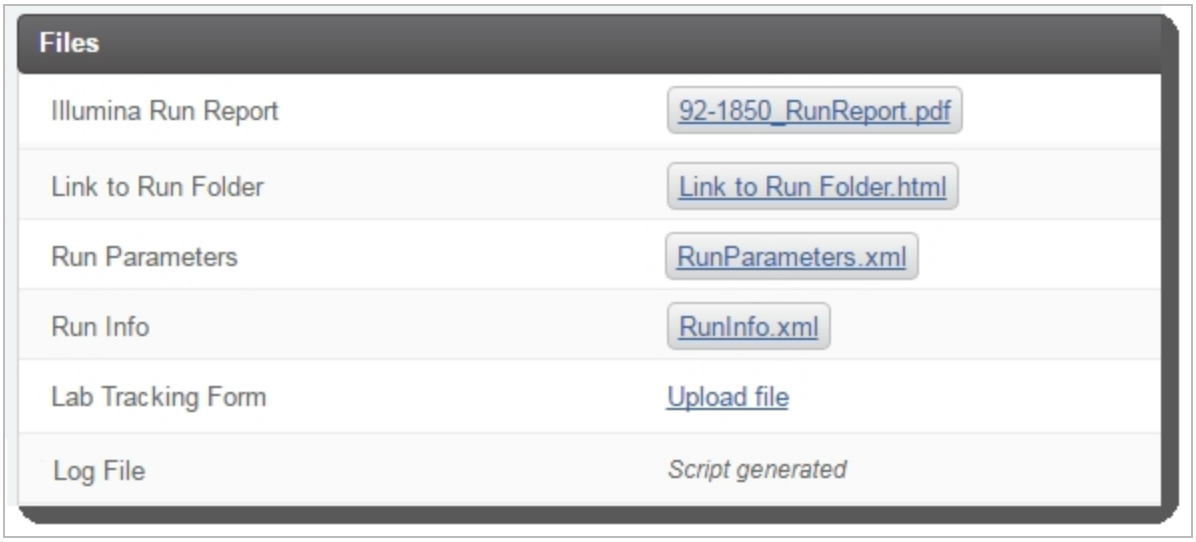
User Interaction

1. The user manually starts the step and leaves it in progress until the sequencing service has finished processing the run and the run report is generated and attached (see below).

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2. On the Record Details screen, the read-only field values are automatically populated as the sequencer runs.
3. Once the run has completed:
    - The read-only fields shown in the screenshot above are populated.
    - The Illumina Run Report is generated and attached.
    - The Link to Run Folder is generated and attached.
    - The Run Parameters and Run Info files are attached.
4. [Optional] In theFiles section, the user uploads the Lab Tracking Form.

    Figure 19 Record Details screen (Files section) of NextSeq 500/550 Run (NextSeq 500/550 v1.0) step

5. The user completes the step. (Note that the Log File is attached once the next step for samples has been assigned by the Next Step - Advanceautomation.) User must not complete the step until the Illumina Run Report has been attached to the step in the LIMS.

Components Installed

Note that this integration requires installation of the Pre-configured Workflow Package (PCWP). For details, see Release Notes.

The Illumina NextSeq 500/550 Integration Package v2.0 RPM installs the scripts and files listed in Table 10. Additional component details are provided in the sections following the table.

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Figure 20 Table 10: Illumina NextSeq 500/550 Integration Package v2.0 - scripts and files installed

Files Installed	Location and Description
configure_extensions_nextseq_sequencingservice.sh	*/opt/gls/clarity/config/* Script that installs the service properties in the database
log4j.xml	*/opt/gls/clarity/extensions/nextseq/SequencingService/conf* File containing the settings for the sequencing jar's logging.
nextseq-sequencing.jar	*/opt/gls/clarity/extensions/nextseq/SequencingService* Jar file containing API-based LIMS extensions used for capturing run results and report generation.
InterOp libraries	*/opt/gls/clarity/extensions/nextseq/lib* Illumina shared library for parsing InterOp data files.
• BCL2FASTQ_Reverse_Complement_Samplesheet.csv • BCL2FASTQ_Samplesheet.csv (Installed by the PCWP)	*/opt/gls/clarity/extensions/nextseq/Templates* Template file used for file generation. In this integration, the reverse complement template is used by default.

Support for Multiple seqservice.netPathPrefixSearch Property Values

It is possible to configure support for multiple, identical seqservice.netPathPrefixSearch property values. For details, see Configuring Multiple Identical netPathPrefixSearch Values.

Database Properties Installed

Table 11 lists the database properties installed with Illumina NextSeq 500/550 Integration v2.0.

Note that several additional properties, each with the '99' suffix appended to their name, are also installed. These are intended for use by the BaseSpace Clarity LIMS support team in automated validation tests and are not listed in the table.

Constraints

Sequencing runs are matched using the reagent cartridge ID and the sequencing step's base name – NextSeq 500/550 Run (NextSeq 500/550 v1.0).

Do not change this name – it is expected by the sequencing service that captures instrument run results. The base name is stored in the sequenceProcessBaseName database property (see Table 11). If this name is changed without the database property being updated, the 'reagent cartridge ID <-> sequencing step base name' matching system will fail.

If required, you may modify the step name by editing or adding to the text after the base name portion as this is not used in the matching system. For example, you could change NextSeq 500/550Run (NextSeq 500/550 v1.0) to NextSeq 500/550Run (NextSeq 500/550 v1.0) v2

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Figure 21 Table 11: Database properties installed with the Illumina NextSeq 500/550 Integration Package

Property	Description	Default Value
nextseq.v2.seqservice.sequenceProcessBaseName *(installed from the PCWP)*	Sequencing process type/master step base display name. Partial matching is used to look up the process type / master step.	NextSeq 500/550 Run (NextSeq 500/550 v1.0)
nextseq.v2.seqservice.eventFileDirectory.1	A network location monitored for event files. E.g., /mnt/illumina/gls_events/'	/mnt/gls_events
nextseq.v2.seqservice.netPathPrefixSearch.1	The network directory prefix contained in the event file - most likely in Windows format.	\\nas\network\run_data
nextseq.v2.seqservice.netPathPrefixReplace.1	The mapped network directory mount name on the server used to access the run data directory. Eg. /mnt/network/data'	/mnt/run_data

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Property	Description	Default Value
nextseq.v2.seqservice.eventFileDirectorySuffixes	A list of eventFileDirectory path entries to monitor for event files. The value is one or more comma separated integers.	99 Configured on install to point to "1"
nextseq.v2.seqservice.netPathPrefixSearchReplaceSuffixes	A list of netPathPrefix search and replace entries for transforming Windows to Linux network paths. The value is one or more comma-separated integers.	99 Configured on install to point to "1"
nextseq.v2.seqservice.runReportViewsVersion	The current version of the Run Report views in the database. The value 0 represents the state before the views are created. This property is automatically updated by the run report.	0

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Property	Description	Default Value
nextseq.v2.seqservice.ignoreUnmatchedContainerIds	A flag indicating if event files that cannot be matched to reagent cartridges in the LIMS should be archived after a certain period of time (true), or continually reprocessed (false).	false To prevent the gls_ events file directory from becoming cluttered, it is recommended that the value of this property is set to 'true'.
nextseq.v2.seqservice.ignoreUnmatchedContainerIds WaitDays	The number of days between when the event is created and the event file is archived.	14
nextseq.v2.seqservice.synchronizationPeriod	Invocation period in seconds.	60

Support for Multiple seqservice.netPathPrefixSearch Property Values

It is possible to configure support for multiple, identical seqservice.netPathPrefixSearch property values. For details, see Configuring Multiple Identical netPathPrefixSearch Values.

Reagent Categories/Label Groups Installed

Reagent categories/label groups are installed with the PCWP workflow configuration slices. For details, see the Reagent Categories / Label Groups article.

Reagent Kits Installed

The following reagent kits are included in the Illumina NextSeq Integration:

- NextSeq Reagent Kit

Control Types Installed

The following control types are included in the Illumina NextSeq Integration:

- PhiX v3

Container Types Installed

The following container types are included in the Illumina NextSeq Integration:

- NextSeq Reagent Cartridge

See also Support for container types.

Instrument Integration

To ensure that your Illumina instrument warranty remains valid, the instrument integration must be performed and maintained by the BaseSpace Clarity LIMS Support team. To perform this integration, the Support team will require remote access to the instrument while it is idle.

Below is an overview of the steps performed by the LIMS support team when configuring the instrument for use with the Illumina NextSeq Integration.

Configuring the Illumina instrument:

1. Create directory on the local computer to hold the batch files. These batch files write event files to the network attached storage (NAS) share(s).
2. Create directory on the NAS to hold the event files.
3. Modify Illumina software configuration files to call the batch files that create the event files.
4. Update sequencing service default database properties to match the specifics of the installation.

Support for Container Types

All one-dimensional container types with both numeric rows and numeric columns are supported.

Rules and Constraints

This integration operates with the following constraints:

- The reagent cartridge ID must be unique. There should not be multiple reagent cartridge containers in the system with the same name.
- The reagent cartridge ID must be scanned as the reagent cartridge Container Name on the Denature and Dilute (NextSeq 500/550 v1.0) step.

Illumina NextSeq 500/550 Integration v2.0 Validation and Troubleshooting

Contents

- Introduction
- Validation steps
- Activate workflow, create project, add and assign samples1. Activate the workflows
  - 2. Create a project, add samples

- Library Prep Validation v1.0 protocolRun Library Prep Validation v1.0 step
- NextSeq 500/550 Sequencing v1.0 protocol1. Run Library Pooling (NextSeq 500/550 v1.0) step
  - 2. Run Denature and Dilute (NextSeq 500/550 v1.0) step
  - 3. Run NextSeq Run (NextSeq 500/550 v1.0) step
- Validating creation of event files
- Troubleshooting

Introduction

This guide explains how to validate the installation of the Illumina NextSeq 500/550 Integration Package v2.0.

The validation process involves:

- Running samples through the Library Prep Validation v1.0 workflow. The workflow contains a single-step protocol that models the library prep required to produce normalized libraries. At the end of the step, the normalized libraries are automatically advanced to the NextSeq 500/550 Sequencing v1.0 workflow.
- Running normalized libraries through the NextSeq 500/550 Sequencingv1.0 workflow. This validates the following:Automated generation of a sample sheet for use with bcl2fastq2 v2.20.0 analysis software.
  - Automated tracking of the NextSeq sequencing run and parsing of run statistics into the LIMS, including:Run status and metrics of sequencing run
  - Sequencing run parameters
  - Real Time Analysis v2 (RTA2) run directory location and other run specific information

Validation Steps

Activate Workflow, Create Project, Add and Assign Samples

The following steps set up the LIMS in preparation for running samples through the Library Prep Validation v1.0 and NextSeq 500/550 Sequencing v1.0 workflows.

1. Activate the Workflows

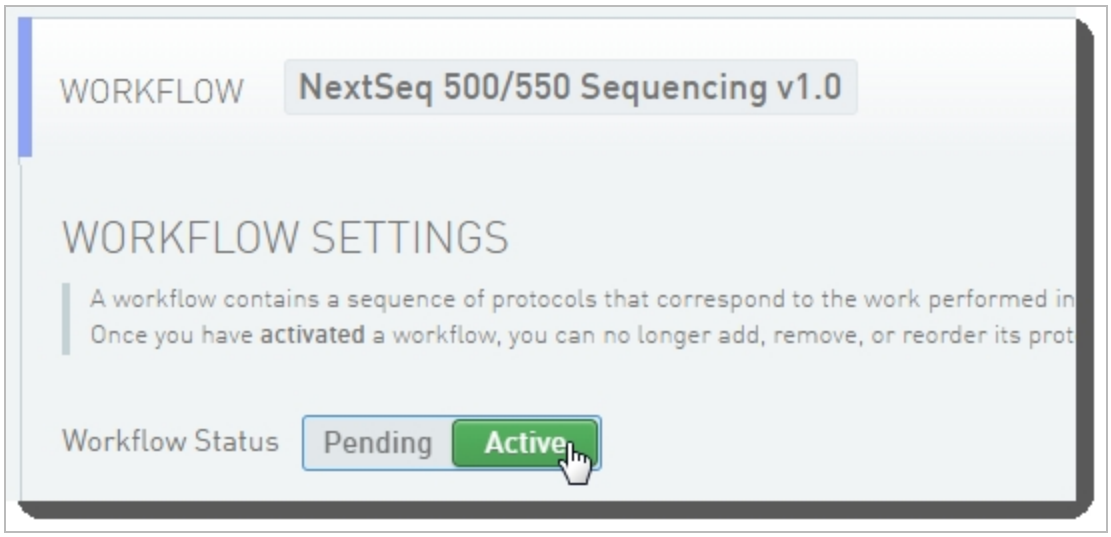
- In the LIMS configuration area, activate the Library Prep Validation v1.0 and NextSeq 500/550 Sequencing v1.0 (shown below) workflows.

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Figure 22 Workflow configuration and activation - LIMS v4.2

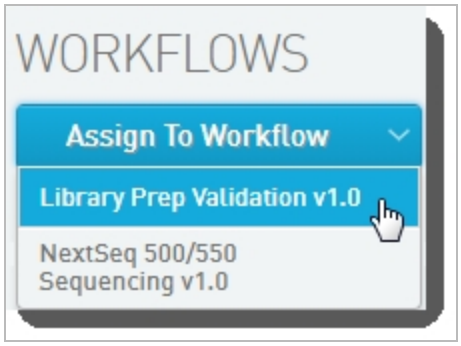


Figure 23 Workflow configuration and activation - LIMS v5.0



2. Create a Project, Add Samples

1. On the Projects and Samples screen, create a project and add samples to it.
2. Assign your samples to the Library Prep Validation v1.0 workflow.



Library Prep Validation v1.0 Protocol

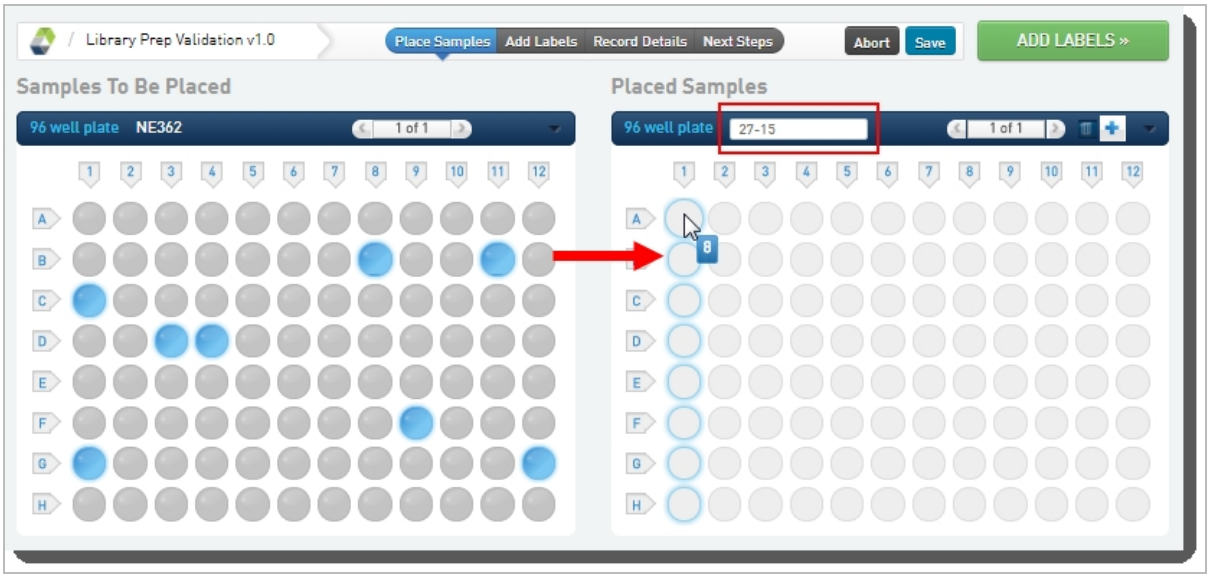
This single-step protocol models the library prep required to produce normalized libraries that are ready for the NextSeq 500/550 Sequencing v1.0 workflow.

Run Library Prep Validation v1.0 Step

1. In Lab View, locate the Library Prep Validation v1.0 protocol. You will see your samples queued for the Library Prep Validation v1.0 step.

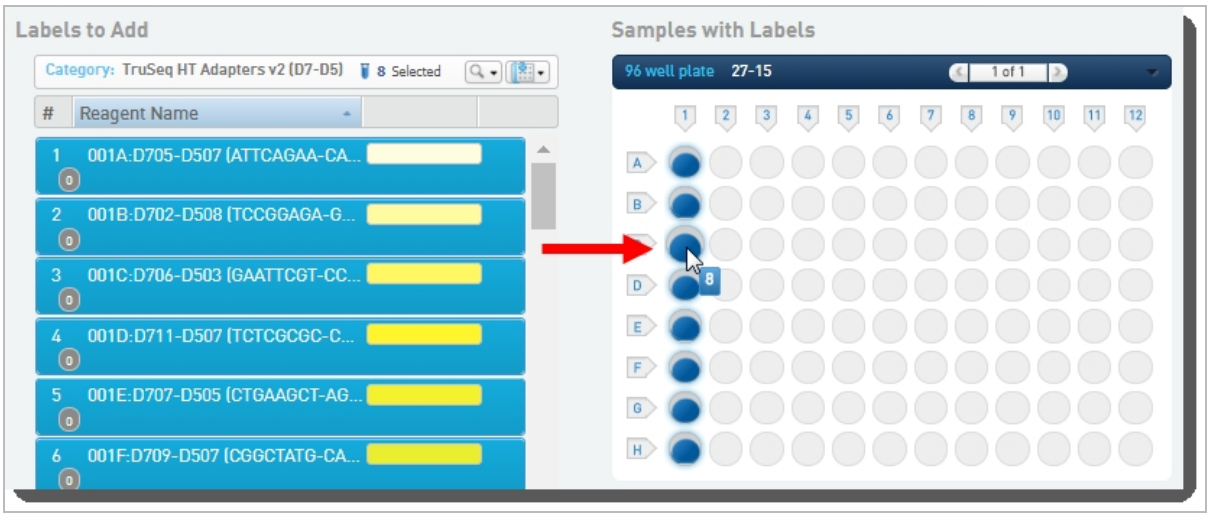


2. Add the samples to the Ice Bucket and click View Ice Bucket.
3. On the Ice Bucket screen, click Begin Work.
4. On the Placement screen:
   a. In the Placed Samples area on the right, in the container field, scan or enter the barcode of the destination container.
   b. Select the samples from the Samples to be Placed area on the left, and then drag them over to the container wells on the right.



   c. Click Add Labels.
5. On the Add Labels screen:
   a. Select labels on the left, and drag them over to the container on the right to assign them to placed samples.

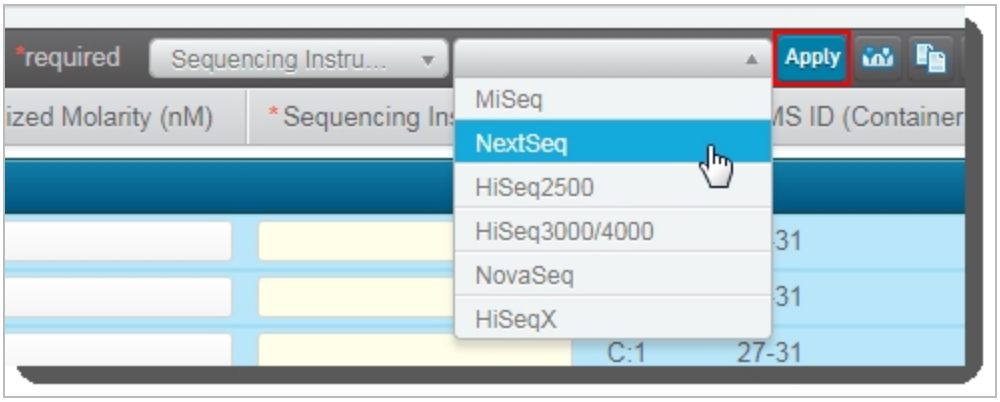
For Research Use Only. Not for use in diagnostic procedures.



b. Click Record Details.

6. On the Record Details screen:

a. Select all of the samples in the Sample Details table and then in the Sequencing Instrument dropdown list, select NextSeq.



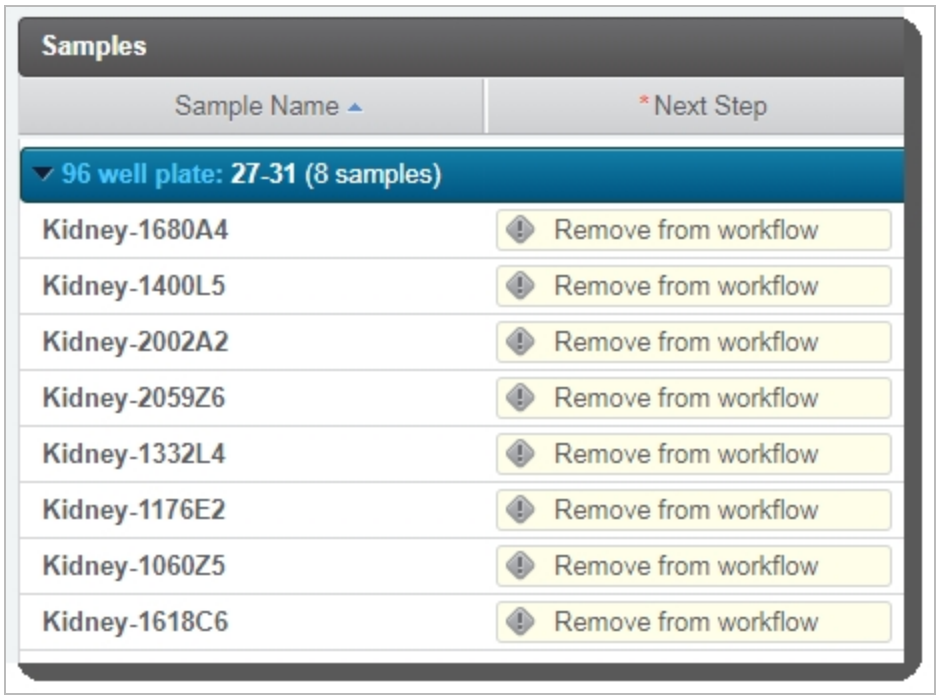
b. Click Apply to set this value for all selected samples.

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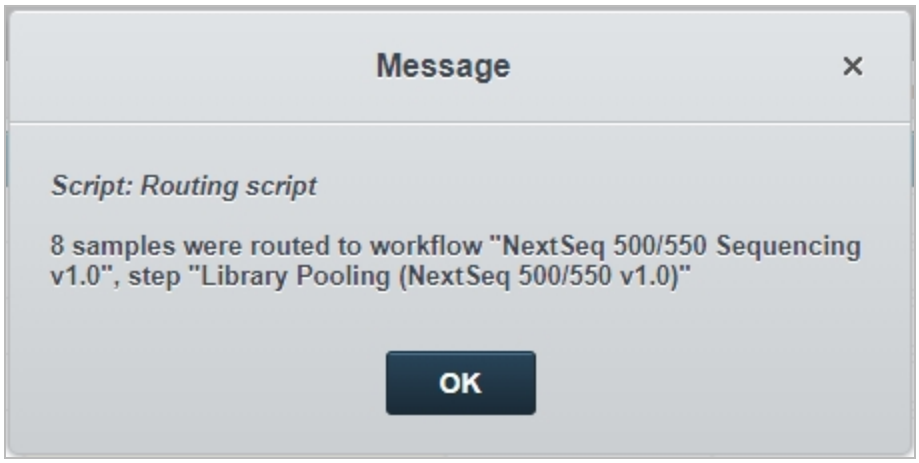
c. Click Next Steps.

7. On the Assign Next Steps screen, the next step is already set to Remove from Workflow (this is required by theRouting scriptautomation).



8. Click Finish Step.The Routing script automation is triggered and samples are automatically routed to the first step in the NextSeq 500/550 Sequencing v1.0 protocol - Library Pooling (NextSeq 500/550 v1.0).

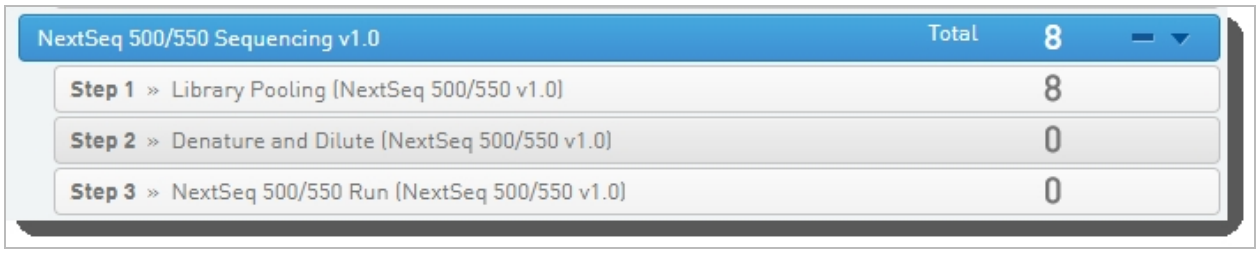
For Research Use Only. Not for use in diagnostic procedures.



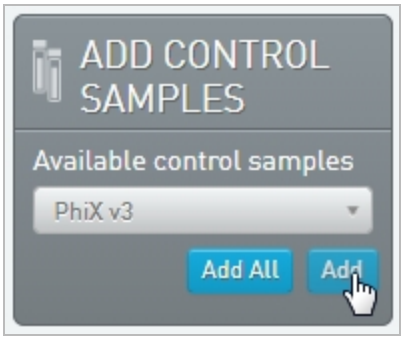
NextSeq 500/550 Sequencing v1.0 Protocol

1. Run Library Pooling (NextSeq 500/550 v1.0) Step

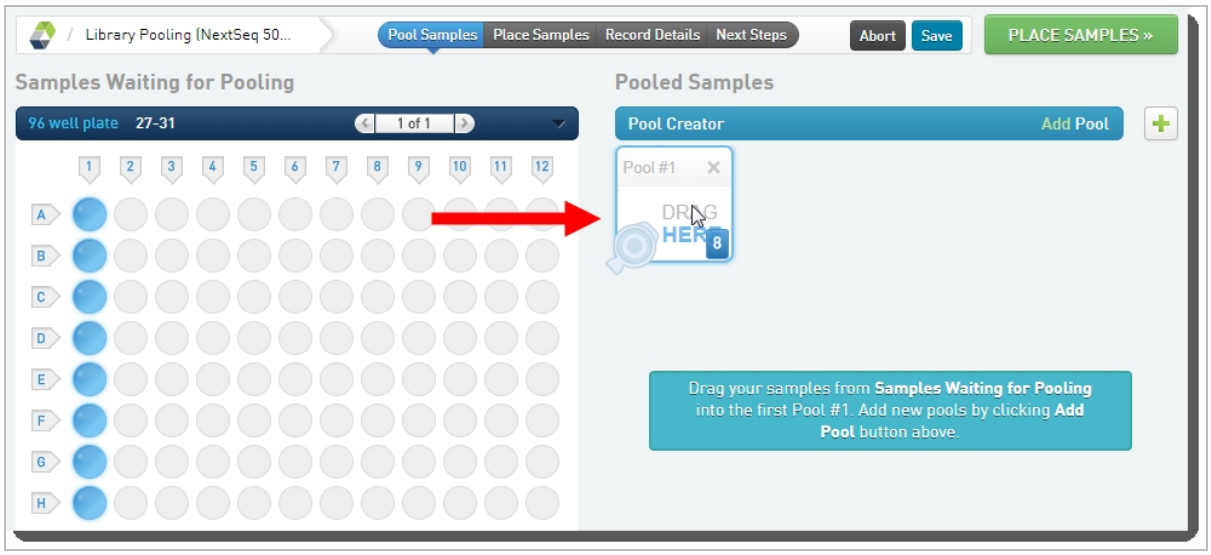
1. In Lab View, locate the NextSeq 500/550 Sequencing v1.0 protocol. You will see your samples queued for the Library Pooling (NextSeq 500/550 v1.0) step. Click the step to proceed to the Queuescreen.



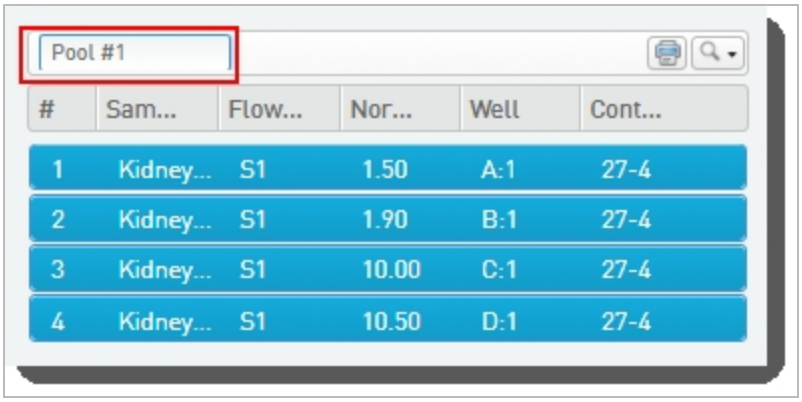
2. On the Queue screen:
   a. Add the samples to the Ice Bucket. In the Add Control Samples panel, add the PhiX v3 control sample to the Ice Bucket.



   b. Click View Ice Bucket.
3. On the Ice Bucket screen, click Begin Work.
4. On the Pool Samples screen:
   a. Create a pool of samples by dragging samples into the Pool Creator.



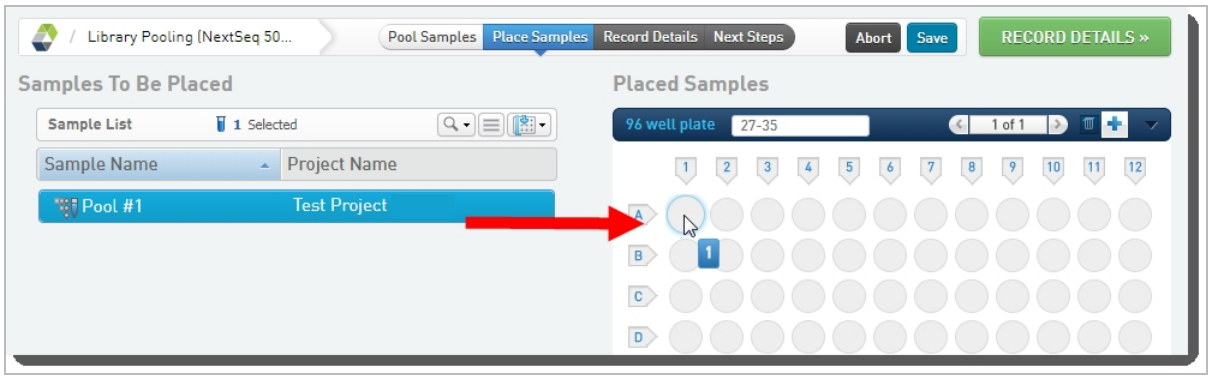
b. Name the pool, or accept the default name - Pool #1.



c. Click Place Samples.

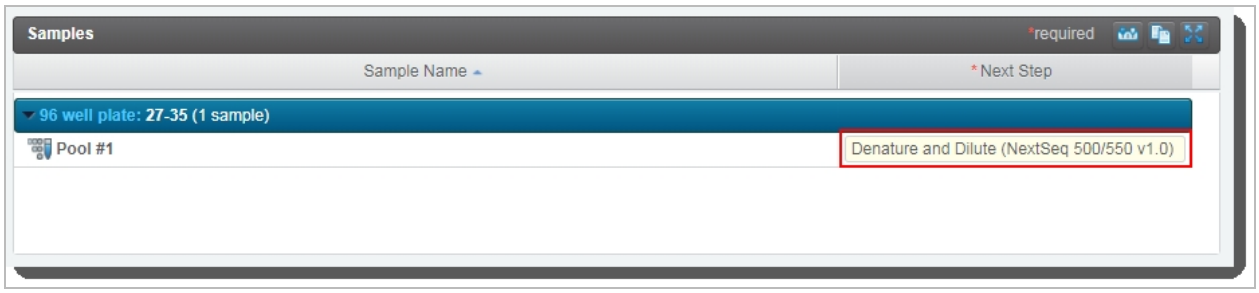
5. On the Placement screen:

a. Select the pool in the Samples to be Placed area on the left and drag it over to the container on the right.



b. Click Record Details.

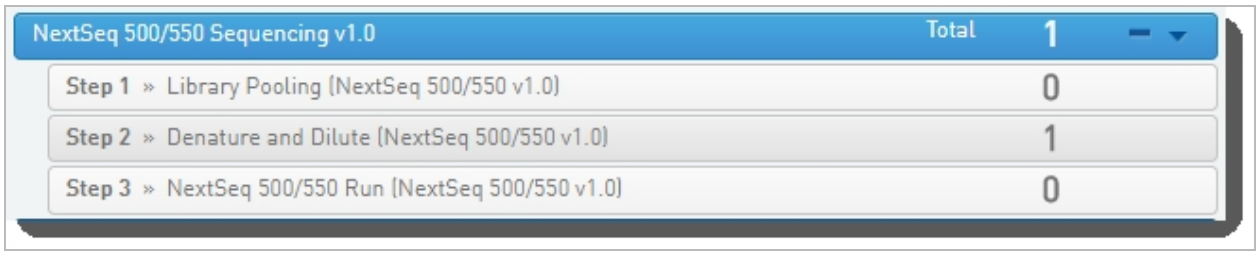
6. On the Record Details screen, click Next Steps.
7. On the Assign Next Steps screen, next step is already set to Denature and Dilute (NextSeq 500/550 v1.0).



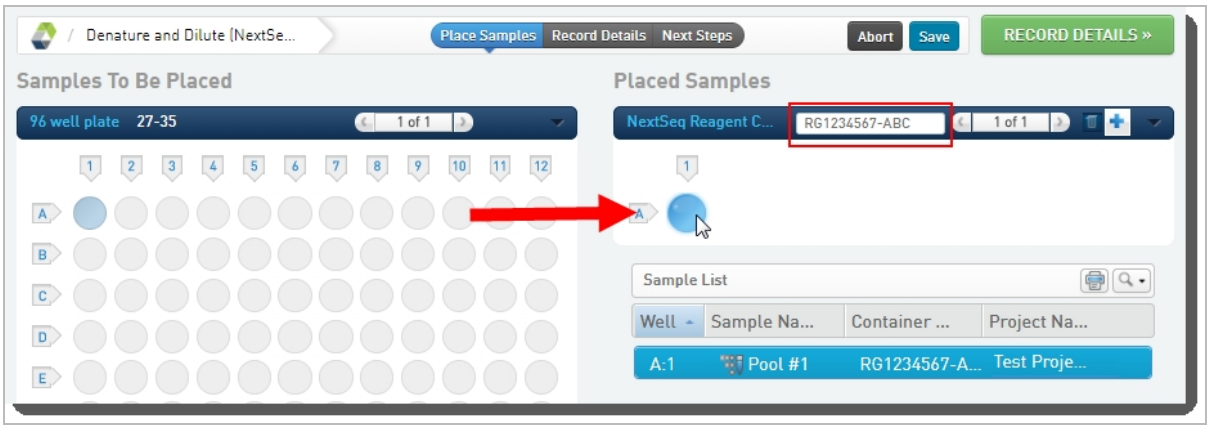
8. Click Finish Step.

2. Run Denature and Dilute (NextSeq 500/550 v1.0) Step

1. In Lab View, locate the NextSeq 500/550 Sequencing v1.0 protocol. You will see your pooled samples queued for the Denature and Dilute (NextSeq 500/550 v1.0) step. Click the step to proceed to the Queuescreen.



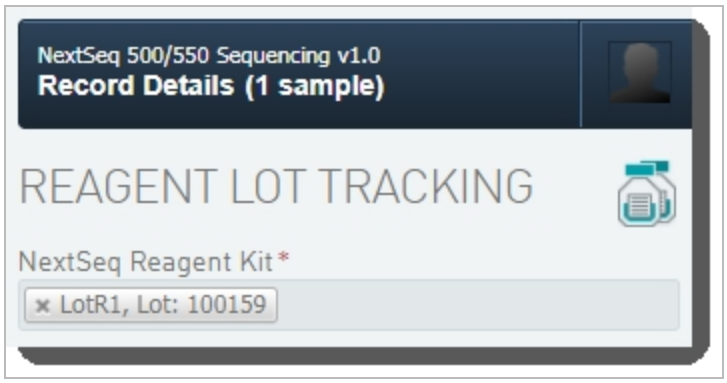
2. On the Queue screen, add the pool to the Ice Bucket and click View Ice Bucket.
3. On the Ice Bucket screen, click Begin Work.
4. On the Placement screen:
   a. Scan the NextSeq reagent cartridge barcode into the NextSeq Reagent Cartridge field.
   b. Place the pool of samples into the reagent cartridge.



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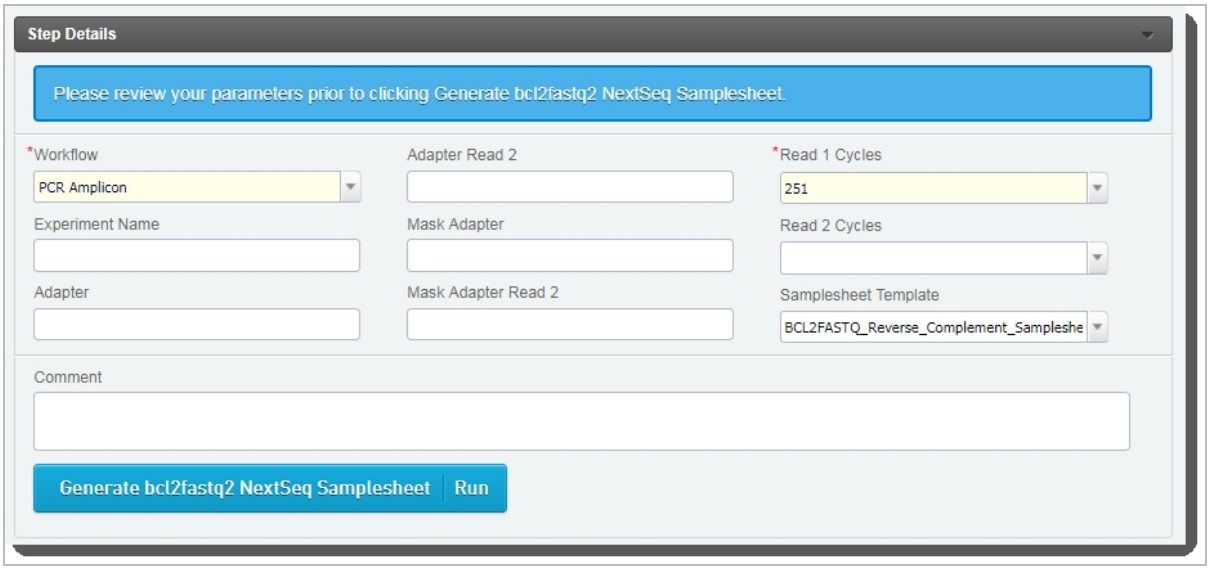
c. Click Record Details.

5. On the Record Details screen:
6. Under Reagent Lot Tracking, select the reagent lot used in the step. (You may need to add/activate the lot on the Reagents and Controlsscreen.)

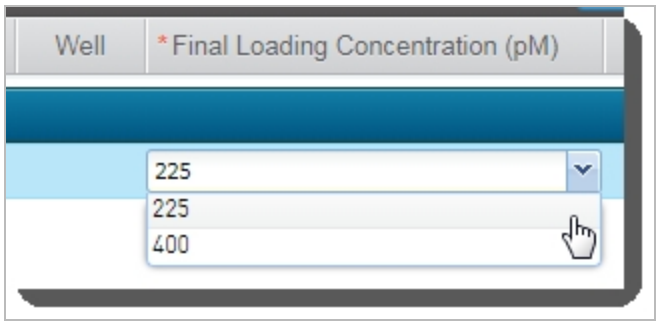


a. In the Step Details table, populate the fields as appropriate. Note the following:Workflow and Read 1 Cycles are required fields.

b. In the Samplesheet Template drop-down list, the reverse complement template is selected by default. Do not change this value.

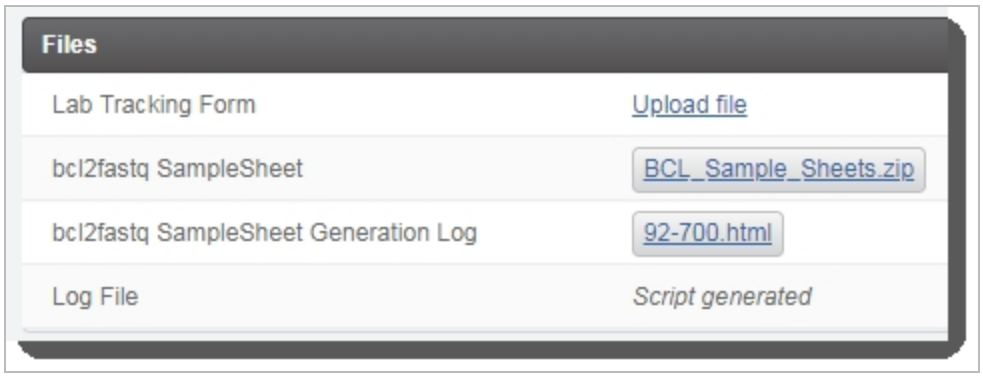


c. In the Sample Details table, enter the Final Loading Concentration. You may select from the two preset options - 225 (for PCR-free workflows) or400(for Nano workflows), or enter a different value.



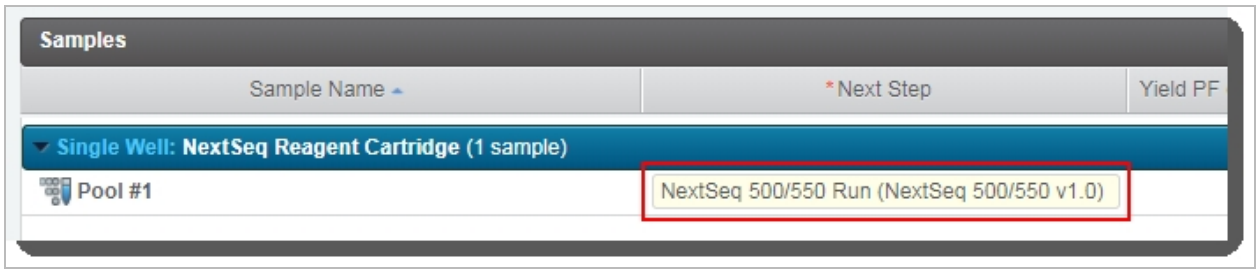
d. Click Generate bcl2fastq2 NextSeq SampleSheet.

e. The LIMS generates the samplesheet and attaches it and a log file to placeholders in the Files area of the Record Detailsscreen. Download the files and validate their format and content.



f. Click Next Steps.

7. On the Assign Next Steps screen, samples are already assigned to the NextSeq 500/550 Run (NextSeq 500/550 v1.0) step.

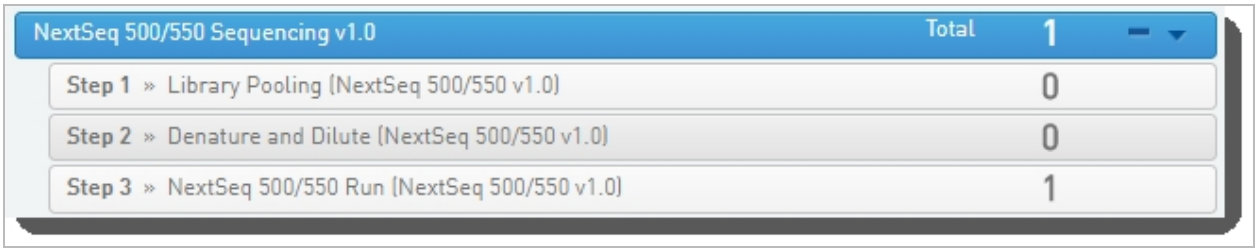


8. Click Finish Step.

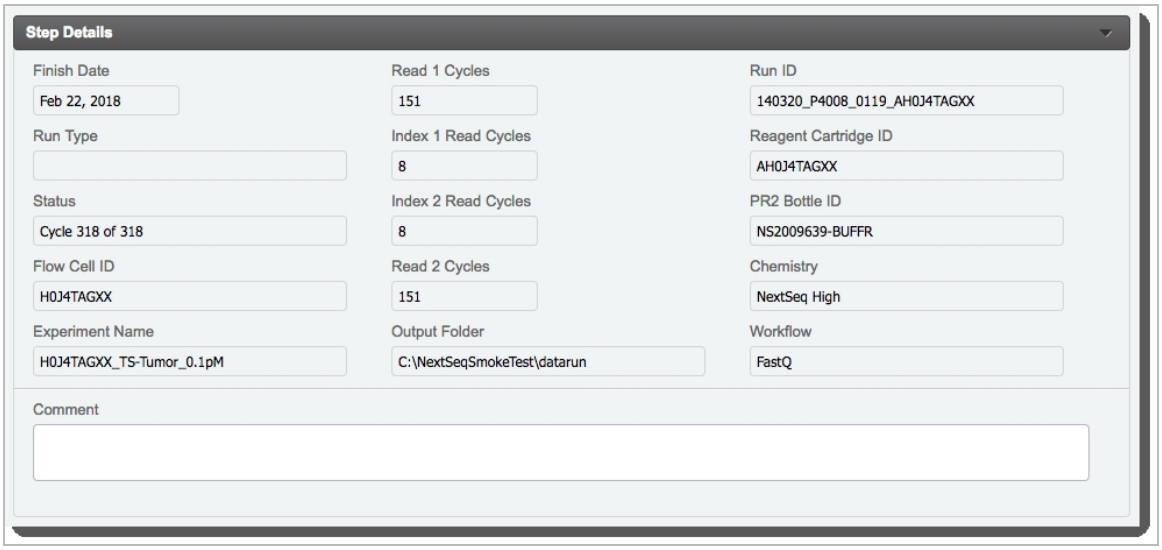
3. Run NextSeq 500/550 Run (NextSeq 500/550 v1.0) Step

1. In Lab View, locate the NextSeq 500/550 Sequencing v1.0 protocol. You will see your pool of samples queued for the NextSeq 500/550 Run (NextSeq 500/550 v1.0) step. Click the step to proceed to the Queuescreen.

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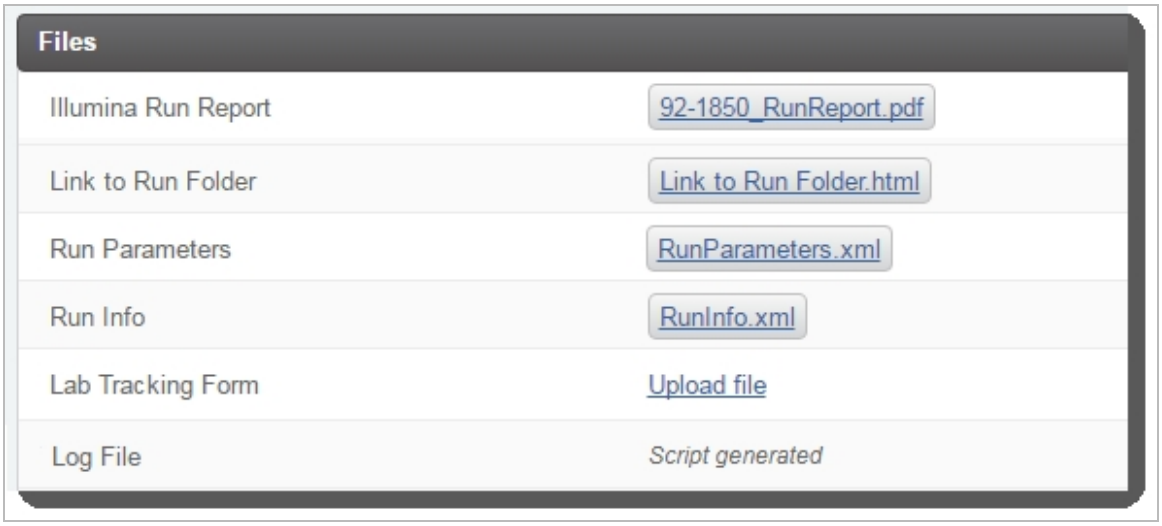


2. Add the pool to the Ice Bucket and click View Ice Bucket.
3. On the Ice Bucket screen, click Begin Work.
4. On the Record Details screen, the fields in the table are read-only. Once the run completes, the integration will automatically:
    - Populate the fields.



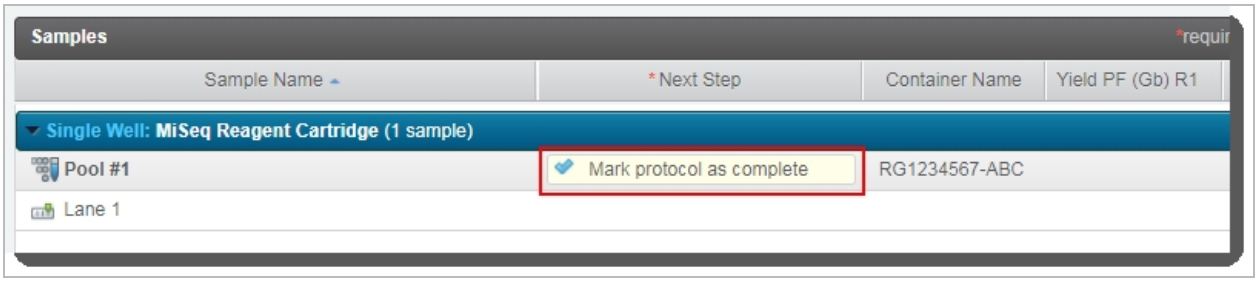
    - Attach files to the Illumina Run Report, Link to Run Folder, Run Parameters, and Run Info placeholders. (Note that the Log File is attached once the next step for samples has been assigned by the Next Step - Advanceautomation.)

      Figure 24 Do not continue to step 5 and complete the step until the Illumina Run Report has been attached.



- Populate the fields in the Sample Details table. See *User and Configuration Guide* for details.

5. Click Next Steps.
6. On the Assign Next Steps screen, the next step is already set to Mark protocol as complete.



7. Click Finish Step.

Validating Creation of Event Files

Follow the steps below to confirm that event files are created by the batch file in the destination path. The steps assume that the default configuration has been successfully imported.

This test validates that:

- Your DESTINATION_PATH is correctly configured.
- The instrument computer can access and write to the DESTINATION_PATH.
- There are no syntax errors in the LIMS batch file.

Steps

1. In C:\gls, double-click gls_event_ncs_rta.bat
2. Confirm that an 'empty' event file appears in the configured DESTINATION_PATH.

Manual invocation of the batch file will produce output resembling the following:

Filename: event-EndRun-07295667.txt

Contents: cycleNumber = runFolder = netFolder = \\eventType = EndRun softwareType = NCSfinishDate = 2018-03-31

Troubleshooting

If an automation trigger does not appear to run its corresponding scripts, see:

- *Troubleshooting Automated Informatics* in the LIMS documentation (Automated Informatics section)
- *Troubleshooting EPP / Automation* in the API > API Overview > API Advanced Topics section

If an error occurs that does not provide direction on how to proceed:

- Confirm the version of the installed Illumina NextSeq Integration Package by running the following command from the server console:

```
rpm -qa | grep -i nextseq
```

If the error is related to data parsing, retrieving run results data, or report values not appearing as expected:

- Review the NextSeqIntegrator.log file, located at /opt/gls/clarity/extensions/Illumina_NextSeq/v2/SequencingService

Additional troubleshooting information for this integration is provided on the Illumina Integrations FAQ page.

If you are unable to resolve the issue, contact the BaseSpace Clarity LIMS Support team, supplying the relevant information from the troubleshooting already performed.

Illumina NextSeq 500/550 Integration v2.0 Release Notes

- Updated configurable SampleSheet generation SampleSheet generation is now done using the updated Template File Generator tool, which enables updates of the provided template for SampleSheet generation in order to add additional headers and columns.
  - Template provided with accompanying configuration generates the BCL2Fastq SampleSheet with reverse complement.
  - When a new custom template is created, it can be uploaded using the Template File Management feature introduced in BaseSpace Clarity LIMS 5.1.
- New delivery mechanism for NextSeq 2.0 Integration workflow configuration NextSeq 500/550 Workflow configuration now delivered using a robust, Preconfigured Workflow Package installer.
  - Install history is recorded with version and install date information for better tracking and troubleshooting of installation.

- Simplified Library Prep step for validationSingle step mock library prep protocol included with configuration to facilitate quick validation of installed NextSeq 2.0 Sequencing Protocol.
- Bugs resolved in this release:Updated the Sequencing Interops Metrics parser to record more accurate metrics using the same parsing libraries as the instrument and SAV.
  - Resolved issue Sequencing Step summary table with incorrect metrics calculated, by using the same parser as instrument.
  - Fixes of various internal automated test issues including smoketests.

NextSeq 1.0 Sample Sheet Generation for bcl2fastq v2.15

Package Version: BaseSpace Clarity LIMS NextSeq Package v1.0.1; bcl2fastq v2.15

New in 1.0.1:

- Configuration changed to use generate_bcl2fastq_sample_sheet script for sample sheet generation.
- Update to the configuration shown in Script usage to make use of new sample sheet options, such as -useSampleLimsID and -appendLimsID.

Contents

- Overview
- Configured Step UDFs
- Submitted Sample UDFs
- Script Parameters
- Script Usage
- Logging
- Sample Sheet Data

Overview

As a part of the NextSeq integration, BaseSpace Clarity LIMS provides the ability to generate a sample sheet from the Denature, Dilute and Load Sample (NextSeq) 1.0 step.

The sample sheet is generated by means of a script, which is initiated when the user clicks a button on Record Details view.

The generated sample sheet is intended to be used with the bcl2fastq conversion tool, provided by Illumina. The tool handles demultiplexing and conversion of the BCL base-call files output by the NextSeq instrument into FastQ files used by many third-party analysis platforms.

With this package, the LIMS provides support for bcl2fastq v2.15. For more information on this product, see Illumina's bcl2fastq User Guide.

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Configured step UDFs

The fields listed in the following table are available on the Denature, Dilute and Load Sample (NextSeq) 1.0 step and will be placed into the sample sheet.

Field Name	Field Type	Required?
Experiment Name	Text	No
Workflow	Text	Yes
Read 1 Cycles	Numeric	No
Read 2 Cycles	Numeric	No
Adapter	Text	No Required if using 'Adapter Read 2'
Adapter Read 2	Text	No
Mask Adapter	Text	No Required if using 'Mask Adapter Read 2'
Mask Adapter Read 2	Text	No

Submitted Sample UDFs

Field Name	Field Type	Required?	Notes
Description	Text	No	See “-l {useProjectLimsID}” command-line parameter. (Script Parameters section)

Script Parameters

-u {username}	LIMS username (Required)
-p {password}	LIMS password (Required)
-i {processURI}	NextSeq sequencing process URI (Required) (lower case I)
-c, {csvFileLimsID}	Sample Sheet CSV file LIMS ID (Required)
-e, {logFileName}	Log file name (Required)
-l, {useProjectLimsID}	Accepted values: true or false. Provide with quotes e.g. -l 'true' (Optional) Project LIMS IDs will be used instead of Project Names in the sample sheet (lower case L)
-s {useSampleLimsID}	Accepted values: true or false. Provide with quotes e.g. -s 'true' (Optional) Sample LIMS IDs will be used instead of Sample Names in the sample sheet

-u {username}	LIMS username (Required)
-a {appendLimsID}	Accepted values: true or false. Provide with quotes e.g. -a 'true' (Optional) The LIMS ID of the step will be appended to the Sample Name values in the sample sheet. Use this option to guarantee unique FASTQ file names per run.

Script Usage

Below is the default command line that ships with the Denature, Dilute and Load Sample (NextSeq) 1.0 step in new installations. The generate_bcl2fastq_sample_sheet script portion of the parameter string is shown in bold.

```
bash -c "/usr/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar script:setUDF -u {username} -p {password} -i {processURI:v2:http} -f 'Progress' -t '//input/@uri-<//sample/@uri' -v 'Library ready for sequencing' && /usr/bin/java -jar /opt/gls/clarity/extensions/Illumina_NextSeq/v1/EPP/nextseq-extensions.jar script:generate_bcl2fastq_sample_sheet -u {username} -p {password} -i {processURI:v2:http} -c {compoundOutputFileLuid1} -e {compoundOutputFileLuid2} "
```

Logging

Info Messages	Reason/Condition
[Info] – Added data line for input: {sample data added for}	For each line of data added to the sample sheet.
[Info] - Successfully generated Illumina MiSeq Sample Sheet. Script added {number of lines added} lines to sheet.	On successful completion with no errors or warnings.

Error Messages	Reason/Condition
Unable to determine {user or password} .	Unable to determine FTP credentials for ftp.user or ftp.password.
Workflow is a required Field.	Workflow is not set.
If you specify Read 2 Cycles, then you must also specify Read 1 Cycles	Read 2 Cycles is set, but Read 1 Cycles is not set.
If you specify Adapter Read 2, then you must also specify Adapter	Adapter Read 2 is set, but Adapter is not set.
If you specify Mask Adapter Read 2, then you must also specify Mask Adapter	Mask Adapter Read 2 is set, but Mask Adapter is not set.
This step only supports a single NextSeq Reagent Cartridge. Please restart the step with a single Cartridge.	More than one output container was found for the step.

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Error Messages	Reason/Condition
Could not find NextSeq Reagent Cartridge RFID. The protocol step doesn't seem to output the correct type.	Cannot find NextSeq Cartridge container type associated with the protocol step.
Could not find a file output. Ensure your EPP string is configured with the correct parameters.	Cannot find a shared result file output whose LIMS ID matches the one provided on the command line for the sample sheet.
All inputs must have a single index. Inputs {name of artifact} has no index.	Encountered a sample without an index when single index is expected.
All inputs must be dual indexed. Input {name of artifact} has no index.	Encountered a sample without an index when dual index is expected.
In a non-indexed run there can only be one input.	Encountered a second sample with no index when the first sample had no index.
Input {name of sample} has an index. Another input was non-indexed. Only one input can be provided on a non-indexed run. On an indexed run, all inputs must be uniquely indexed.	Encountered a sample with an index after already encountering a sample with no index.
Unable to find reagent type {reagent label} for input {name of artifact}.	Cannot find a reagentType that matches the label on a sample.
Duplicate index found on input {name of sample}. All inputs must have a unique index.	Encountered a sample with a duplicate index.
All inputs must have a single index. Input {name of sample} has a dual index.	Encountered a sample with a dual index when we expect all samples to have a single index.
All inputs must have a dual index. Input {name of sample} has a single index.	Encountered a sample with a single index when we expect all samples to have a dual index.

Sample Sheet Data

Below is a listing of all fields that can appear in the sample sheet and the fields that are populated.

Only items in bold are used by the bcl2fastq tool when processing. Other fields will be ignored by the tool and are there for your convenience.

Field Name	Required?	Populated by	Comments
[Header]			
Investigator Name	No	LIMS technician name	
Experiment Name	No	Step UDF	
Date	No	EPP run date	
Workflow	Yes	Step UDF	

Field Name	Required?	Populated by	Comments
[Reads]			
Read 1 Cycles	No	Step UDF	
Read 2 Cycles	No	Step UDF	
[Settings]			
Adapter	No*	Step UDF	Required if AdapterRead 2 is used.
AdapterRead 2	No	Step UDF	
MaskAdapter	No*	Step UDF	Required if MaskAdapterRead 2 is used.
MaskAdapterRead 2	No	Step UDF	
[Data]			
SampleID	No	Sample LIMS ID	
SampleName	No	Sample Name	Will be sample name or LIMS ID, depending on command-line value. An additional command line option allows the step LIMS ID to be appended to the end of this value, e.g. "Sample1-1234". (See Script parameters).
SamplePlate	No	Sample input plate	
SampleWell	No	Sample input well location	
index	No	Sample reagent label	Dual index reagents will contain a hyphen-separated DNA sequence. This field will use the first half of that value.
index2	No	Sample secondary reagent label	Dual index reagents will contain a hyphen-separated DNA sequence. This field will use the second half of that value.

Field Name	Required?	Populated by	Comments
Project	No	Sample project Will be name or LIMS ID of the project for the first sample in the sample sheet (see Script parameters).	
Description	No	Sample UDF	Sample UDF

Manually Creating NCS EndRun Event Files

Use the following information to manually create the NextSeq Control Software (NCS) EndRun event files.

Note that BaseSpace Clarity LIMS currently only supports the EndRun event for NCS.

The gls_event_ncs_rta.bat file can be found on the NextSeq Sequencer in c:\gls\

Source Data

RunInfo.xml

- [totalNumberOfCycles] - sum of NumCycles from <Reads> information gleaned from RunInfo.xml.

RunParameters.xml

The following variables are found in the RunParameters.xml file:

- <TempFolder>
- <OutputFolder>

Pattern

```
gls_event_ncs_rta.bat [totalNumberOfCycles] "<TempFolder>" "<OutputFolder>"
```

Example Command Line

```
gls_event_ncs_rta.bat 16 "D:\Illumina\NextSeq Control Software Temp"
"D:\Illumina\NextSeqOutput"
```

Example Output

File name:

- event-EndRun-12555475.txt

For Research Use Only. Not for use in diagnostic procedures.

File contents:

```
cycleNumber = 16runFolder = "D:\Illumina\NextSeq Control Software Temp\140320_
P4008_0119_AH0J4TAGXX"netFolder = "D:\Illumina\NextSeqOutput\120208_M99999_0009_
ARG1234567-00CDE"eventType = EndRunsoftwareType = NCS
```

Illumina NextSeq Instrument Interface

To ensure that your Illumina instrument warranty remains valid, this integration must be performed and maintained by the BaseSpace Clarity LIMS Support team as needed.

Overview of the BaseSpace Clarity LIMS ↔ Illumina NextSeq Instrument Interface

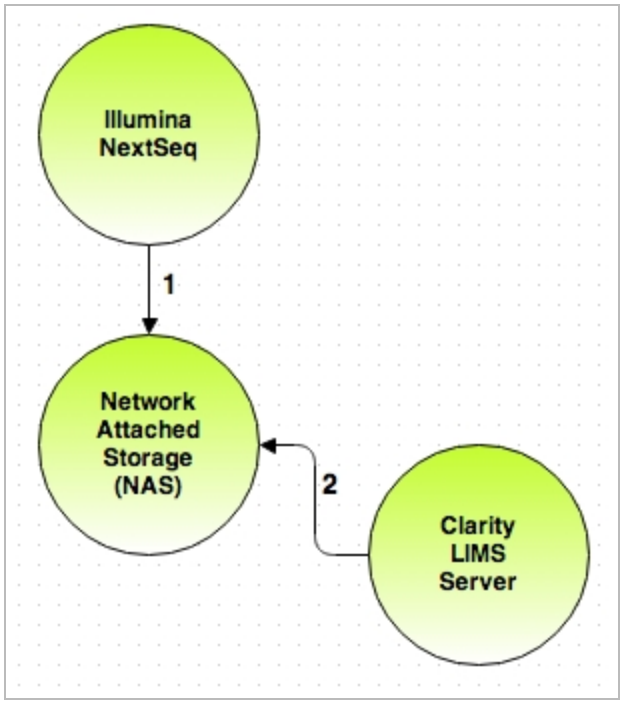


Image Action #	Description
0	NextSeq Run (NextSeq) 1.0 initiated in the LIMS.
1	1. End Run event fires on Illumina instrument. 2. Instrument fires configured LIMS script. 3. Script writes End Run event to NAS network root folder in \gls_events .

Image Action #	Description
2	1. Timer-based daemon on the LIMS server wakes up and looks for events. 2. Daemon discovers End Run event and processes it. 3. Daemon uses the supplied process run directory to locate the run folder on the NAS. 4. Daemon parses key information from the run folder including the Reagent Cartridge ID. 5. Daemon uses LIMS REST API to do the following: – Populate step UDFs: Finish Date, Run Type, Status, Flowcell ID, Experiment Name, Read 1 Cycles, Index 1 Read Cycles, Index 2 Read Cycles, Read 2 Cycles, Output Folder, Run ID, Reagent Cartridge ID, PR2 Bottle ID, Chemistry, Workflow – Examine list of files to collect, and attach any that are available: – RunInfo.xml, RunParameters.xml, Link to Run Folder.html – Run statistics are parsed from several binary InterOp files and stored (visible in the NextSeq run). – Illumina Run Report invoked, generated, and attached.

iScan Integrations

Te Illumina iScan integration provides preconfigured workflows that map to established lab protocols and steps used with the iScan system. The integration also supports the associated consumables and assays used in these protocols.

iScan Integration v1.1 BeadChip Accessioning, Imaging, and Analysis

The Illumina iScan Integration Package v1.1 supports the integration of Clarity LIMS to iScan systems. The package includes the following workflows, which are included in the Illumina Preset Protocols (IPP) Package v2.6:

- iLASS Infinium Genotyping Assay v1.0
- iLASS Infinium Genotyping Assay with PGx v1.0

This integration enables you to do the following tasks:

- Accession the BeadChip.
- Handle the imaging run event (including pre-imaging verification for the imaging step).
- Handle the analysis run event for the analysis step.

> The configuration used in this integration supports iScan lab processes. Any changes to the configuration (including renaming protocols, steps, and fields) can cause issues with these processes.

Terminology

As of Clarity LIMS v5, the term user-defined field (UDF) has been replaced with custom field in the user interface. However, the API resource is still called UDF.

There are two types of custom fields:

- Master step fields—Configured on master steps. Master step fields only apply to the following:
  - The master step on which the fields are configured.
  - The steps derived from those master steps.
- Global fields—Configured on entities (eg, submitted sample, derived sample, measurement, etc.). Global fields apply to the entire Clarity LIMS system.

Accession the BeadChip

In the iScan workflows, BeadChips are accessioned into Clarity LIMS before the hybridization step of the post-lab protocol. The BeadChip container type is defined based on the BeadChip product type (eg, PGx) and the product version. Because every BeadChip has a unique barcode, Clarity LIMS can track usage throughout the entire workflow. The following BeadChip names are supported in iScan Integration v1.1:

- GCRA-24v1-0
- GCRAPGx-24v1-0
- GDA_PGx-8v1-0
- GSA-24v4-0
- GSA-48v4-0
- GSAPGx-24v4-0
- GSAPGx-48v4-0

If you need help accessioning your BeadChip, contact Illumina Support.

Image Infinium BeadChip on iScan Step

Master Step: Image Infinium BeadChip on iScan (iLASS Infinium Genotyping Assay v1.0) or Image Infinium BeadChip on iScan (iLASS Infinium Genotyping Assay with PGx v1.0)

This step is automated and must not be started manually.

In this step, the iScan Integration handles the following iScan imaging process events:

- Pre-imaging verification
- Imaging run started
- Run completed
- Run aborted
- Run error

Master Step Custom Fields

The following table lists field configuration details defined on the Image Infinium BeadChip on iScan (iLASS Infinium Genotype Assay/with PGx v1.0) master step. These fields are located under Configuration, on the Master Step Fields tab.



Do not delete, rename, or modify. These actions will break the integration.

Field Name	Field Type	Options	Additional Options and Dropdown Items
BeadChip Barcode	Text	Required = No Read Only = Yes	None (automatically populated when imaging is started)
Instrument	Text	Required = No Read Only = Yes	None (automatically populated when imaging is started)
Run Start Time	Text	Required = No Read Only = Yes	None (automatically populated when imaging is started)
Data Path	Text	Required = No Read Only = Yes	None (hidden and automatically populated when imaging is started)
Instrument Run ID	Text	Required = No Read Only = Yes	None (automatically populated when imaging is started)
Run Status	Text	Required = No Read Only = Yes	None (automatically populated when imaging is started, completed, aborted, or has failed)
DMAP Location	Text	Required = No Read Only = Yes	None (hidden and automatically populated when imaging is started)
Instrument Type	Text	Required = No Read Only = Yes	None (automatically populated when imaging is started)
Failure Reason	Text	Required = No Read Only = Yes	None (automatically populated when imaging fails)

Field Name	Field Type	Options	Additional Options and Dropdown Items
Log	Multiline Text	Required = No Read Only = Yes	None (automatically populated when imaging is started, completed, aborted, or has failed)
ICA Run ID	Text	Required = No Read Only = Yes	None (hidden and automatically populated when imaging is started)
Run Complete Time	Text	Required = No Read Only = Yes	None (automatically populated when imaging is completed or aborted)

Pre-Imaging Verification

When BeadChips are loaded onto the iScan system, the Control Software (CS) with LIMS mode enabled performs pre-imaging verification to make sure that user loads the correct BeadChip. This verification is done as follows.

1. CS queries the Illumina Connected Analytics (ICA) GMS service to make sure that the correct BeadChip is loaded. The service continues to poll for the status at a specific interval.
2. Clarity LIMS receives an event from the ICA GMS service on the BeadChip that must be verified and performs the verification.
3. When the BeadChip is verified to be queued to the imaging step and is ready for imaging, Clarity LIMS updates the GMS service that the verification is successful.
4. After CS receives the verification complete status from the GMS service, BeadChip imaging starts automatically.

Imaging Run Status Tracking

When the BeadChip is scanned with an iScan, Clarity LIMS receives the events of the run status update. Then, the custom fields are automatically populated with information.

The Run Status field can show the following statuses:

- Running (for run started)
- Completed (for run completed)
- Aborted (for run aborted)

- Failed (run error events)

If there is a run error event, the Run Complete Time field is not populated.

Invoke Infinium Analysis Step

Master Step: Invoke Infinium Analysis (iLASS Infinium Genotyping Assay v1.0) or Invoke Infinium Analysis (iLASS Infinium Genotyping Assay with PGx v1.0)

In this step, the iScan Integration handles events related to BeadChip analysis. This step is a manual step that requires you to input certain information (eg, Analysis Configuration ID and Analysis Run Name) to start the analysis run.

Master Step Custom Fields

The following table lists field configuration details defined on the Invoke Infinium Analysis (iLASS Infinium Genotyping Assay/with PGx v1.0) step. These fields are located under Configuration, on the Master Step Fields tab.

Field Name	Field Type	Options	Additional Options and Dropdown Items
Analysis Complete Time	Text	Required = No Read Only = Yes	None (automatically populated when analysis is completed or aborted)
Analysis Run Name	Text	Required = Yes Read Only = No	None
Analysis Configuration ID	Text	Required: Yes Read Only: No	None
Analysis Start Time	Text	Required = No Read Only = Yes	None (automatically populated when analysis is started)
Analysis Report	Text	Required = No Read Only = Yes	None (hidden, automatically populated when analysis is completed)

Field Name	Field Type	Options	Additional Options and Dropdown Items
Analysis Status	Text	Required = No Read Only = Yes	None (automatically populated when analysis is started, completed, aborted, or has failed)
Analysis Run ID	Text	Required = No Read Only = Yes	None (hidden, automatically populated when analysis is started)
Log	Multiline Text	Required = No Read Only = Yes	None (automatically populated when analysis is started, completed, aborted, or has failed)

Configure and Start an Analysis Run

1. On the Record Details milestone, navigate to Step Details and enter the Analysis Run Name.
   The Analysis Run Name can only contain a maximum of 255 alphanumeric characters, underscores, hyphens, and periods. The name cannot start with the *wfr.* characters.
2. Enter the Analysis Configuration ID.
   To retrieve the configuration ID that is associated with the analysis, contact Illumina Support.
3. Select Start Analysis v1.0 to start the analysis run.

Analysis Run Status Tracking

When the analysis is started on ICA, Clarity LIMS receives the event and displays related information. The custom fields are automatically populated with information.

The Analysis Status field can show the following statuses:

- Analysis Running
- Analysis Completed
- Analysis Completed with Warning
- Analysis Aborted
- Analysis Timedout
- Analysis Errored

If there is an analysis error event, the Analysis Complete Time field is not populated.

iScan Integration v1.1 Release Notes

These Release Notes describe the key changes to software components for the Clarity LIMS iScan Integration Package version 1.1.

Compatibility

This version of the integration package is compatible with the following software:

- Clarity LIMS v6.2 or later
- SIS Core Package v2.4.0 or later
- Illumina Preset Protocols (IPP) v2.6.0 or later
- Control Software (CS) v4.2.1

New Features

- Updates to the Java and third-party dependency libraries used in the integration.

Defects Fixed

- Fixed the SIS core service not being properly registered in systemctl.

Known Issues

- Warning log message displays when the ImagingAborted and ImagingFailed events are processed.

iScan Integration v1.0 BeadChip Accessioning, Imaging, and Analysis

The Illumina iScan Integration Package v1.0 supports the integration of Clarity LIMS to iScan systems. The package includes the following workflows, which are included in the Illumina Preset Protocols (IPP) Package v2.4:

- iLASS Infinium Genotyping Assay v1.0
- iLASS Infinium Genotyping Assay with PGx v1.0

This integration enables you to do the following tasks:

- Accession the BeadChip.
- Handle the imaging run event (including pre-imaging verification for the imaging step).
- Handle the analysis run event for the analysis step.

> The configuration used in this integration supports iScan lab processes. Any changes to the configuration (including renaming protocols, steps, and fields) can cause issues with these processes.

Terminology

As of Clarity LIMS v5, the term user-defined field (UDF) has been replaced with custom field in the user interface. However, the API resource is still called UDF.

There are two types of custom fields:

- Master step fields—Configured on master steps. Master step fields only apply to the following:
  - The master step on which the fields are configured.
  - The steps derived from those master steps.
- Global fields—Configured on entities (eg, submitted sample, derived sample, measurement, etc.). Global fields apply to the entire Clarity LIMS system.

Accession the BeadChip

In the iScan workflows, BeadChips are accessioned into Clarity LIMS before the hybridization step of the post-lab protocol. The BeadChip container type is defined based on the BeadChip product type (eg, PGx) and the product version. Because every BeadChip has a unique barcode, Clarity LIMS can track usage throughout the entire workflow. The following BeadChip names are supported in iScan Integration v1.0:

- GDA_PGx-8v1-0
- GSA-48v4-0
- GSAPGx-48v4-0
- GSA-24v4-0
- GCRA-24v1-0
- GSAPGx-24v4-0
- GCRAPGx-24v1-0

If you need help accessioning your BeadChip, contact Illumina Support.

Image Infinium BeadChip on iScan Step

Master Step: Image Infinium BeadChip on iScan (iLASS Infinium Genotyping Assay v1.0) or Image Infinium BeadChip on iScan (iLASS Infinium Genotyping Assay with PGx v1.0)

This step is automated and must not be started manually.

In this step, the iScan Integration handles the following iScan imaging process events:

- Pre-imaging verification
- Imaging run started
- Run completed
- Run aborted

- Run error

Master Step Custom Fields

There are 12 fields defined on the Image Infinium BeadChip on iScan (iLASS Infinium Genotype Assay/with PGx v1.0) master step. These fields are located under Configuration, on the Master Step Fields tab. The following table lists field configuration details.

Field Name	Field Type	Options	Additional Options and Dropdown Items
BeadChip Barcode	Text	Required = No Read Only = Yes	None (automatically populated when imaging is started)
Instrument	Text	Required = No Read Only = Yes	None (automatically populated when imaging is started)
Run Start Time	Text	Required = No Read Only = Yes	None (automatically populated when imaging is started)
Data Path	Text	Required = No Read Only = Yes	None (hidden)
Instrument Run ID	Text	Required = No Read Only = Yes	None (automatically populated when imaging is started)
Run Status	Text	Required = No Read Only = Yes	None (automatically populated when imaging is started, completed, aborted, or has failed)
DMAP Location	Text	Required = No Read Only = Yes	None (hidden)
Instrument Type	Text	Required = No Read Only = Yes	None (automatically populated when imaging is started)
Failure Reason	Text	Required = No Read Only = Yes	None (automatically populated when imaging fails)

Field Name	Field Type	Options	Additional Options and Dropdown Items
Log	Multiline Text	Required = No Read Only = Yes	None (automatically populated when imaging is started, completed, aborted, or has failed)
ICA Run ID	Text	Required = No Read Only = Yes	None (hidden)
Run Complete Time	Text	Required = No Read Only = Yes	None (automatically populated when imaging is completed or aborted)

Pre-Imaging Verification

When BeadChips are loaded onto the iScan system, the Control Software (CS) (CS) with LIMS mode enabled performs pre-imaging verification to make sure that user loads the correct BeadChip. This verification is done as follows.

1. CS queries the ICA GMS service to make sure that the correct BeadChip is loaded. The service continues to poll for the status at a specific interval.
2. Clarity LIMS receives an event from the ICA GMS service on the BeadChip that must be verified and performs the verification.
3. When the BeadChip is verified to be queued to the imaging step and is ready for imaging, Clarity LIMS updates the GMS service that the verification is successful.
4. After CS receives the verification complete status from the GMS service, BeadChip imaging starts automatically.

Imaging Run Status Tracking

When the BeadChip is scanned with an iScan, Clarity LIMS receives the events of the run status update. Then, the custom fields are automatically populated with information. The following fields are updated for the imaging events:

- Run Status
- Log
- Run Complete Time

The Run Status field can show the following statuses:

- Running (for run started)

- Completed (for run completed)
- Aborted (for run aborted)
- Failed (run error events)

If there is a run error event, the Run Complete Time field is not populated.

Invoke Infinium Analysis Step

Master Step: Invoke Infinium Analysis (iLASS Infinium Genotyping Assay v1.0) or Invoke Infinium Analysis (iLASS Infinium Genotyping Assay with PGx v1.0)

In this step, the iScan Integration handles events related to BeadChip analysis. This step is a manual step that requires you to input certain information (eg, Analysis Configuration ID and Analysis Run Name) to start the analysis run.

Master Step Custom Fields

There are eight custom fields defined on the Invoke Infinium Analysis (iLASS Infinium Genotyping Assay/with PGx v1.0) step. These fields are located under Configuration, on the Master Step Fields tab. The following table lists field configuration details.

Field Name	Field Type	Options	Additional Options and Dropdown Items
Analysis Complete Time	Text	Required = No Read Only = Yes	None (automatically populated when analysis is completed or aborted)
Analysis Run Name	Text	Required = Yes Read Only = No	None
Analysis Configuration ID	Text	Required: Yes Read Only: No	None
Analysis Start Time	Text	Required = No Read Only = Yes	None (automatically populated when analysis is started)
Analysis Report	Text	Required = No Read Only = Yes	None (hidden, automatically populated when analysis is completed)

Field Name	Field Type	Options	Additional Options and Dropdown Items
Analysis Status	Text	Required = No Read Only = Yes	None (automatically populated when analysis is started, completed, aborted, or has failed)
Analysis Run ID	Text	Required = No Read Only = Yes	None (hidden, automatically populated when analysis is started)
Log	Multiline Text	Required = No Read Only = Yes	None (automatically populated when analysis is started, completed, aborted, or has failed)

Configure and Start an Analysis Run

1. On the Record Details milestone, navigate to Step Details and enter the Analysis Run Name.
   The Analysis Run Name can only contain a maximum of 255 alphanumeric characters, underscores, hyphens, and periods. The name cannot start with the *wfr.* characters.
2. Enter the Analysis Configuration ID.
   To retrieve the configuration ID that is associated with the analysis, contact Illumina Support.
3. Select Start Analysis v1.0 to start the analysis run.

Analysis Run Status Tracking

When the analysis is started on ICA, Clarity LIMS receives the event and displays related information. The custom fields are automatically populated with information. The following fields are updated for the analysis events:

- Analysis Complete Time
- Analysis Report (this field is hidden)
- Analysis Status
- Log

The Analysis Status field can show the following statuses:

- Analysis Running
- Analysis Completed

- Analysis Completed with Warning
- Analysis Aborted
- Analysis Timedout
- Analysis Errored

If there is an analysis error event, the Analysis Complete Time field is not populated.

iScan Integration v1.0 Release Notes

These Release Notes describe the key changes to software components for the Clarity LIMS iScan Integration Package version 1.0.

Compatibility

This version of the integration package is compatible with the following software:

- BaseSpace Clarity LIMS v5.3 or later
- SIS Core Package v2.3.0 or later
- Illumina Preset Protocols (IPP) v2.4.0 or later
- Control Software (CS) (CS) v4.2.1

New Features

Features provided with this release of the integration package are as follows.

- Integration of ILASS Infinium Genotyping Assay v1.0 and ILASS Infinium Genotyping Assay with PGx v1.0 workflows for the iScan instrument.

Defects Fixed

None

Known Issues

None

Documentation

For detailed information related to this release, refer to *iScan Integration v1.0 BeadChip Accessioning, Imaging, and Analysis* on page 1962.

MiSeq Integrations

The Illumina MiSeq instrument integration provides preconfigured workflows that map to established lab protocols and steps used with the MiSeq instrument. The integration also supports the associated library prep kits, reagent kits, and assays used in these protocols.

MiSeq Integration v8.2 Configuration and User Interaction

The Illumina MiSeq Integration Package v8.2 supports the integration of Clarity LIMS to Illumina MiSeq Sequencing Systems.

The integration allows for automated tracking of an Illumina sequencing run in Clarity LIMS. This capability includes tracking sequencing run status, generating run report, and capturing and parsing run statistics. In addition, this integration provides automated generation of a sample sheet file for use with the MiSeq Control Software (MCS) and Local Run Manager (LRM).

Overview

The Illumina MiSeq Integration Package v8.2 includes the following features:

- Preconfigured MiSeq Sequencing v3.2 workflow that maps to lab protocol steps and sequencing runs.
- Automated generation of a sample sheet for use with the MiSeq Control Software (MCS) and LRM.
- Supported LRM analysis modules are GenerateFastQ, Library QC, Resequencing, DNA Enrichment, and DNA Amplicon.
- Automated tracking of the MiSeq sequencing run and parsing of run statistics into Clarity LIMS, including:
  - Run status and metrics of sequencing run
  - Sequencing run parameters
  - Real-Time Analysis (RTA) v1 run directory location and other run-specific information

This integration also includes a preconfigured Library Prep Validation workflow that is used for validation purposes only. The workflow contains a single-step protocol that models the library prep required to produce normalized libraries that are ready for the MiSeq Sequencing v3.2 workflow. For details, refer to *MiSeq Integration v8.2 Validation and Troubleshooting* on page 2002

The MiSeq Integration Package v8.2 only supports Clarity LIMS v6.2.

The following sections describe the MiSeq Sequencing v3.2 workflow.

For instructions on validating and troubleshooting the Illumina MiSeq Integration Package, refer to *MiSeq Integration v8.2 Validation and Troubleshooting* on page 2002.

Compatibility

- The MiSeq Sequencing v3.2 workflow is only compatible with MiSeq Integration Package v8.2.
- MiSeq Integration v8.2 is only required for MiSeq Control Software (MCS) v4.0. For MCS v3.1 or earlier, do not upgrade to MiSeq Integration v8.2. This upgrade breaks the integration.

Prerequisites and Assumptions

Before samples are assigned to the MiSeq Sequencing v3.2 workflow, make sure that the following prerequisites are completed:

- Samples have been accessioned into Clarity LIMS.
- Samples have been run through QC and library prep.
- Samples have been normalized, and the value is captured in a field called Normalized Molarity (nM).

For more information on sample accessioning, refer to *Sample Accessioning* and *Upload and Modify Samples* in the Clarity LIMS Core Functionality documentation.

Samples can be assigned to the MiSeq Sequencing v3.2 workflow automatically using a routing script or manually from the Projects & Samples dashboard. Refer to *Assign and Process Samples* in the Clarity LIMS Core Functionality documentation.

Workflows, Protocols, and Steps

The Illumina MiSeq Integration includes the MiSeq Sequencing v3.2 workflow, which contains a single protocol of the same name.

The protocol includes the following steps and automations.

Table 405 MiSeq Sequencing v3.2 Protocol Steps and Automations

Step	Automation
1. Library Pooling (MiSeq v3.2)	Validate Maximum Number of Samples Set Next Step - Advance
2. Denature and Dilute (MiSeq v3.2)	Validate Single Input Validate Run Setup and Generate MiSeq SampleSheet Set Next Step - Advance
3. MiSeq Run (MiSeq v3.2)	Set Next Step - Advance

Step 1: Library Pooling (MiSeq v3.2)

In this step, the lab scientist manually places libraries into pools in the Clarity LIMS Placement screen.

Set Next Step - Advance Automation

This automation advances samples to the next step in the protocol. The automation is automatically triggered on exit of the Record Details screen.

The default command line is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-0extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-t false \
-h false \
-exp 'nextStep = ::ADVANCE::' \
-log {compoundOutputFileLuid0}"
```

Validate Maximum Number of Samples Automation

This automation checks the maximum number of samples that is allowed in a single pool. The default maximum for Illumina MiSeq Control Software (MCS) is 1536 samples. The automation is automatically triggered on entry of the Pooling screen.

The default command line is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
script:validateSampleCount -min 1 -max 1536"
```

Master Step Fields

The following field is configured on the Library Pooling (MiSeq v3.2) master step. The field displays on the Record Details screen at run time.

Table 406 Library Pooling (MiSeq v3.2) Master Step Field Configuration

Field Name	Field Type	Field Constraints/Options
Comment	Multiline Text	Not applicable

Global Fields

The following table lists the global fields that are displayed on the Queue and Ice Bucket screens of the Library Pooling (MiSeq v3.2) step. Most fields display in expanded view only.

Table 407 Global Field Configuration for Library Pooling (MiSeq v3.2)

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Configured on submitted sample:			
Application	Text Dropdown	Custom Entries	Presets • TruSeq mRNA sequencing • TruSeq DNA sequencing (large genome *de novo*) • TruSeq DNA sequencing (large genome re-seq) • TruSeq DNA sequencing (small genome *de novo*) • TruSeq DNA sequencing (small genome re-seq) • Nextera DNA sequencing • TruSeq Custom Amplicon sequencing • ChIP-sequencing • Exome sequencing • Mate pair sequencing • Small RNA sequencing
Pooling	Text Dropdown	Custom Entries	Presets • Yes • No
Read Length	Text		Not applicable
Sequencing Coverage	Text		Not applicable

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Sequencing Method	Text Dropdown	Custom Entries	Presets • Single Read • Paired End Read • Indexed Single Read • Indexed Paired End Read
Configured on derived sample:			
Normalized Molarity (nM)	Numeric		Decimal places displayed = 2

User Interaction

1. On the Queue screen, do as follows.
   a. Add the samples to the Ice Bucket.
   b. [Optional] In the Add Control Samples panel, add the PhiX v3 control sample to the Ice Bucket.
   c. Select View Ice Bucket.
2. On the Ice Bucket screen, select Begin Work.
   The Validate Maximum Number of Samples automation script runs. The maximum number of samples allowed is 1536.
3. On the Pool Samples screen, do as follows.
   a. Create a pool of samples by dragging samples into the Pool Creator.
   b. Name the pool or accept the default name (Pool #1).
   c. Select Place Samples.
4. On the Placement screen, do as follows.
   a. Select the pool in the Samples to be Placed area and drag it to the container.
   b. Select Record Details.
5. On the Record Details screen, select Next Steps.
   On the Assign Next Steps screen, next step is already set to Denature and Dilute (MiSeq v3.2).
6. Select Finish Step.

Step 2: Denature and Dilute (MiSeq v3.2)

In this step, pooled libraries are denatured and diluted, and then placed into the reagent cartridge loaded into the MiSeq instrument.

Validate Single Input Automation

This automation checks that there is only one pooled input in the step. The automation is automatically triggered when starting the step.

The default command line is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:validateSampleCount -min 1 -max 1"
```

Validate Run Setup and Generate MiSeq SampleSheet Automation

This automation validates the information entered on the Record Details screen, generates the sample sheet (refer to *Sample Sheet Generation*), and attaches the sample sheet to the Denature and Dilute step.

Select a button on the Record Details screen to trigger this automation.

Validation Rules

- The Experiment Name custom field cannot exceed 40 characters.
- The Experiment Name field can only contain letters, numbers, periods, nonconsecutive spaces, and the following special characters: `~!@#%-_}{.
- If Starling is selected as the variant caller, the Export to gVCF field must be set to Yes.
- If the Read 2 Cycles field is empty or 0, the following rules apply:
  - The Custom Primers field cannot have a selection that contains Read 2.
  - The Adapter Read 2 field cannot have a value.
  - The UMI - Read 2 Length field and the UMI - Read 2 Start From Cycle field cannot have values.
- The UMI - Read 1 Length field and the UMI - Read 1 Start From Cycle field cannot be used independently.
- The UMI - Read 2 Length field and the UMI - Read 2 Start From Cycle field cannot be used independently.
- If the UMI - Read 2 Length field is greater than 0, the UMI - Read 1 Length field must be greater than 0.
- The Adapter Read 1 and Adapter Read 2 fields can only contain ACGT+ characters. The adapter sequence cannot start or end with + or contain more than one +.
- For DNA Enrichment analysis, if the Variant Caller value is Somatic, the Variant Frequency Percentage must be between 1 and 100.
- For DNA Amplicon analysis, if the Variant Caller value is Somatic, the Variant Frequency Percentage field must contain a value between 0.05 and 30.

- If the Aligner value is BWA or TruSeq Amplicon Aligner, an error occurs when the Workflow value is not equal to DNA Amplicon.
- If the Aligner value is BWA-MEM or BWA-Backtrack Legacy, an error occurs when the Workflow value is equal to DNA Amplicon.
- If the Variant Caller value is Starling or GATK, an error occurs when the Workflow value is equal to DNA Amplicon.
- If the Variant Caller value is Somatic, an error occurs when the Workflow value is not equal to DNA Enrichment or DNA Amplicon.
- If the Variant Caller value is Germline, an error occurs when the Workflow value is not equal to DNA Amplicon.

The default command line is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-exp {udf:Validation Script 1} {udf:Validation Script 2}'\
-log {compoundOutputFileLuid3} \
-t true \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/MiSeqSamplesheetv3.csv \
-o {compoundOutputFileLuid1}.csv \
-q true \
-destLIMSID {compoundOutputFileLuid1} \
-l {compoundOutputFileLuid2}"
```

There is an 8000 character limit in the Oracle database used for the automation storage. Because of this character limit, the automation splits and saves the validation expressions to the Validation Script 1 and Validation Script 2. These fields are configured as master step fields.

Validation Script 1 Command Line

```
if (step.::Experiment Name::.length() > 40) { fail(::Experiment Name cannot
exceed 40 characters.::) }; if (!step.::Experiment Name::.matches(::^(?!.*[ ]
{2})[a-zA-Z0-9-_`.~!#@%{ }]+::)) { fail(::Experiment Name contains prohibited
characters. Allowed characters are letters, numbers, periods, non-consecutive
spaces and the following special characters: `~!@#%-_}{::) }; if
(step.::Workflow:: == ::Resequencing:: && step.::Variant Caller:: ==
::Starling:: && (!step.hasValue(::Export to gVCF::) || step.::Export to gVCF::
```


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```
!= ::Yes::)) { fail(::Export to gVCF must be set to Yes for Starling variant
caller.::) }; if (step.::Workflow:: == ::DNA Enrichment:: && step.::Variant
Caller:: == ::Somatic:: && (!step.hasValue(::Variant Frequency Percentage::) ||
step.::Variant Frequency Percentage:: > 100 || step.::Variant Frequency
Percentage:: < 1)) { fail(::In the Variant Frequency Percentage field, please
enter values between 1 and 100.::) }; if (step.::Workflow:: == ::DNA Amplicon::
&& step.::Variant Caller:: == ::Somatic:: && (!step.hasValue(::Variant Frequency
Percentage::) || step.::Variant Frequency Percentage:: > 30 || step.::Variant
Frequency Percentage:: < 0.05)) { fail(::In the Variant Frequency Percentage
field, please enter values between 0.05 and 30.::) }; if (!step.hasValue(::Read
2 Cycles::) || step.::Read 2 Cycles:: == 0) { if (step.::Custom
Primers::.contains(::Read 2::)) { fail(::Custom Primers setting selected is
invalid and can only be used in a Paired-End run.::) }; if (step.hasValue
(::Adapter Read 2::)) { fail(::Adapter Read 2 is only applicable for a Paired-
End run.::) }; if (step.hasValue(::UMI - Read 2 Length::) || step.hasValue(::UMI
- Read 2 Start From Cycle::)) { fail(::UMI - Read 2 Length and UMI - Read 2
Start From Cycle are only applicable for a Paired-End run.::) }; }; if
(step.hasValue(::UMI - Read 1 Length::) && !step.hasValue(::UMI - Read 1 Start
From Cycle::)) { fail(::UMI - Read 1 Start From Cycle must be greater than 0 if
UMI - Read 1 Length is greater than 0.::) }; if (!step.hasValue(::UMI - Read 1
Length::) && step.hasValue(::UMI - Read 1 Start From Cycle::)) { fail(::UMI -
Read 1 Length must be greater than 0 if UMI - Read 1 Start From Cycle is greater
than 0.::) }; if (step.hasValue(::UMI - Read 2 Length::) && !step.hasValue(::UMI
- Read 2 Start From Cycle::)) { fail(::UMI - Read 2 Start From Cycle must be
greater than 0 if UMI - Read 2 Length is greater than 0.::) }; if
(!step.hasValue(::UMI - Read 2 Length::) && step.hasValue(::UMI - Read 2 Start
From Cycle::)) { fail(::UMI - Read 2 Length must be greater than 0 if UMI - Read
2 Start From Cycle is greater than 0.::) }; if (!step.hasValue(::UMI - Read 1
Length::) && step.hasValue(::UMI - Read 2 Length::)) { fail(::UMI - Read 1
Length must be greater than 0 if UMI - Read 2 Length is greater than 0.::) }; if
(step.hasValue(::Adapter Read 1::) && !step.::Adapter Read 1::.matches(::^
[ACGT]+(\\+[ACGT]+){0,1}$::)) { fail(::Adapter Read 1 contains prohibited
characters. Allowed characters are: ACGT+ and the adapter sequence cannot start
or end with + or contain more than one +.::) }; if (step.hasValue(::Adapter Read
2::) && !step.::Adapter Read 2::.matches(::^[ACGT]+(\\+[ACGT]+){0,1}$::)) { fail
(::Adapter Read 2 contains prohibited characters. Allowed characters are: ACGT+
and the adapter sequence cannot start or end with + or contain more than one
+.::) };
```

Validation Script 2 Command Line

```
if ((step.::Aligner:: == ::BWA:: || step.::Aligner:: == ::TruSeq Amplicon
Aligner::) && step.::Workflow:: != ::DNA Amplicon::) { fail(::Aligner field
contains invalid value. Please refer to the \u005c\u0022Applicable analysis
fields for the selected Workflow:\u005c\u0022 section for more information.::)
}; if ((step.::Aligner:: == ::BWA-MEM:: || step.::Aligner:: == ::BWA-Backtrack
Legacy::) && step.::Workflow:: == ::DNA Amplicon::) { fail(::Aligner field
contains invalid value. Please refer to the \u005c\u0022Applicable analysis
fields for the selected Workflow:\u005c\u0022 section for more information.::)
}; if ((step.::Variant Caller:: == ::Starling:: || step.::Variant Caller:: ==
::GATK::) && step.::Workflow:: == ::DNA Amplicon::) { fail(::Variant Caller
field contains invalid value. Please refer to the \u005c\u0022Applicable
analysis fields for the selected Workflow:\u005c\u0022 section for more
information.::) }; if (step.::Variant Caller:: == ::Somatic:: &&
step.::Workflow:: != ::DNA Enrichment:: && step.::Workflow:: != ::DNA
Amplicon::) { fail(::Variant Caller field contains invalid value. Please refer
to the \u005c\u0022Applicable analysis fields for the selected
Workflow:\u005c\u0022 section for more information.::) }; if (step.::Variant
Caller:: == ::Germline:: && step.::Workflow:: != ::DNA Amplicon::) { fail
(::Variant Caller field contains invalid value. Please refer to the
\u005c\u0022Applicable analysis fields for the selected Workflow:\u005c\u0022
section for more information.::) }; step.::Indel-Realignment-Key:: = ::::;
step.::Indel-Realignment-Value:: = ::::; step.::Aligner-Key:: = ::::;
step.::Aligner-Value:: = ::::; step.::Variant-Caller-Value:: = ::::; if
(step.::Workflow:: == ::DNA Amplicon::) { if (step.::Indel Realignment:: ==
::Yes::) { step.::Indel-Realignment-Key:: = ::variantcallerrealignindels::;
step.::Indel-Realignment-Value:: = ::1::; }; else if (step.::Indel Realignment::
== ::No::) { step.::Indel-Realignment-Key:: = ::variantcallerrealignindels::;
step.::Indel-Realignment-Value:: = ::0::; }; if (step.::Aligner:: == ::TruSeq
Amplicon Aligner::) { step.::Aligner-Key:: = ::aligner::; step.::Aligner-Value::
= ::Amplicon::; }; else if (step.::Aligner:: == ::BWA::) { step.::Aligner-Key::
= ::aligner::; step.::Aligner-Value:: = ::BWA::; }; }; if (step.::Workflow:: !=
::DNA Amplicon::) { if (step.::Indel Realignment:: == ::Yes::) { step.::Indel-
Realignment-Key:: = ::indelrealignment::; step.::Indel-Realignment-Value:: =
::GATK::; }; else if (step.::Indel Realignment:: == ::No::) { step.::Indel-
Realignment-Key:: = ::indelrealignment::; step.::Indel-Realignment-Value:: =
::None::; }; if (step.::Aligner:: == ::BWA-Backtrack Legacy::) { step.::Aligner-
Key:: = ::runbwaaln::; step.::Aligner-Value:: = ::1::; }; else if
(step.::Aligner:: == ::BWA-MEM::) { step.::Aligner-Key:: = ::runbwaaln::;
step.::Aligner-Value:: = ::0::; }; }; if (step.hasValue(::Variant Caller::)) {
if (step.::Variant Caller:: == ::None::) { step.::Variant-Caller-Value:: = ::::;
```


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```
}; if (step.::Variant Caller:: == ::GATK::) { step.::Variant-Caller-Value:: =
::GATK::; }; else if (step.::Variant Caller:: == ::Starling::) { step.::Variant-
Caller-Value:: = ::Starling::; }; else if (step.::Variant Caller:: ==
::Germline::) { step.::Variant-Caller-Value:: = ::PiscesGermline::; }; else if
(step.::Variant Caller:: == ::Somatic:: && step.::Workflow:: == ::DNA
Amplicon::) { step.::Variant-Caller-Value:: = ::PiscesSomatic::; }; else if
(step.::Variant Caller:: == ::Somatic:: && step.::Workflow:: != ::DNA
Amplicon::) { step.::Variant-Caller-Value:: = ::Somatic::; }; }; if
(step.hasValue(::Manifest::)) { step.::Manifest-Section:: = ::[Manifests]::; };
else { step.::Manifest-Section:: = ::::; }
```

Set Next Step - Advance Automation

This automation advances samples to the next step in the protocol. The automation is automatically triggered by exiting the Record Details screen.

The default command line is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-t false \
-h false \
-exp 'nextStep = ::ADVANCE::' \
-log {compoundOutputFileLuid3}"
```

Master Step Fields

Most fields configured on the Denature and Dilute (MiSeq v3.2) step display on the Record Details screen in the Step Data table.

These fields are manually populated at run time (refer to *User Interaction* on page 1990). The values are then used to generate the sample sheet.

Table 408 Denature & Dilute (MiSeq v3.2) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Drop-Down Items
Adapter Read 1	Text		Not applicable
Adapter Read 2	Text		Not applicable

Field Name	Field Type	Options	Additional Options and Drop-Down Items
Aligner	Text Dropdown		Presets • None (default) • BWA-MEM • BWA-Backtrack Legacy • BWA • TruSeq Amplicon Aligner
Aligner-Key	Text	Read Only	Hidden, used for sample sheet generation Do not delete / modify this field. These actions will break sample sheet generation automation.
Aligner-Value	Text	Read Only	
Annotation	Text Dropdown		Presets • None (default) • RefSeq • Ensembl
Applicable analysis fields for the selected Workflow	Multiline Text	Read Only	Select a value from the drop-down list in the upper-right corner (below NEXT STEPS).
Comment	Multiline Text		Not applicable

Field Name	Field Type	Options	Additional Options and Drop-Down Items
Custom Primers	Text Dropdown		Presets • None (Default) • Read 1 • Index • Read 2 • Read 1, Index • Read 1, Index, Read 2 • Read 1, Read 2 • Index, Read 2
Description	Text		Not applicable
Experiment Name	Text	Required Field	Not applicable
Export to gVCF	Text Dropdown		Presets • None (default) • Yes • No
Flag PCR Duplicates	Text		Presets • None (default) • Yes • No
Genome Folder	Text		Not applicable
Indel Realignment	Text Dropdown		Presets • None (default) • Yes • No
Indel Repeat Filter Cutoff	Text Dropdown		Presets • None (default) • Yes • No

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Field Name	Field Type	Options	Additional Options and Drop-Down Items
Indel-Realignment-Key	Text	Read Only	Hidden, used for sample sheet generation Do not delete / modify this field. These actions will break sample sheet generation automation.
Indel-Realignment-Value	Text	Read Only	
Manifest	Text		Not applicable
Manifest Padding	Text Dropdown		Presets • None (default) • 0 • 50 • 100 • 150 • 200 • 250
Manifest-Section	Text	Read Only	Hidden, used for sample sheet generation Do not delete / modify this field. These actions will break sample sheet generation automation.
Picard HS Metric Reporting	Text Dropdown		Presets • None (default) • Yes • No

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Field Name	Field Type	Options	Additional Options and Drop-Down Items
Read 1 Cycles	Numeric Dropdown	Required Field Custom Entries	Presets • 251 (Default) • 151 • 101 • 76 • 51 Range = 1–1000 Decimal places displayed = 0
Read 2 Cycles	Numeric Dropdown	Custom Entries	Presets • 251 • 151 • 101 • 76 • 51 Range = 0–1000 Decimal places displayed = 0
Read Stitching	Text Dropdown		Presets • None (default) • Yes • No
Reverse Complement	Text Dropdown		Presets • None (default) • Yes • No
UMI - Read 1 Length	Numeric		Minimum value: 1
UMI - Read 1 Start From Cycle	Numeric		Minimum value: 1
UMI - Read 2 Length	Numeric		Minimum value: 1
UMI - Read 2 Start From Cycle	Numeric		Minimum value: 1

Field Name	Field Type	Options	Additional Options and Drop-Down Items
Validation Script 1	Multiline Text	Required Field Read Only	Hidden, used for automation Do not delete / modify this field. These actions will break sample sheet generation automation.
Validation Script 2	Multiline Text	Required Field Read Only	
Variant Caller	Text Dropdown		Presets • None (Default) • GATK • Germline • Somatic • Starling
Variant Caller Depth Filter	Numeric		Minimum value: 10 Maximum value: 10000
Variant Quality Filter	Numeric		Minimum value: 2 Maximum value: 1000
Variant Frequency Percentage	Numeric		Minimum value: 0.05 Maximum value: 100 Decimal places displayed = 2
Variant-Caller-Value	Text	Read Only	Hidden, used for sample sheet generation Do not delete / modify this field. These actions will break sample sheet generation automation.

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Field Name	Field Type	Options	Additional Options and Drop-Down Items
Workflow	Text Dropdown	Required Field	Presets • GenerateFASTQ • LibraryQC • Resequencing • DNA Enrichment • DNA Amplicon

Groups of Defaults

The following groups of defaults are configured for Denature and Dilute (MiSeq v3.2) step fields:

Resequencing

Field	Configuration
Workflow	Resequencing
Read 1 Cycles	251
Custom Primers	None
Aligner	BWA-MEM
Annotation	None
Export to gVCF	No
Flag PCR Duplicates	Yes
Genome Folder	Required
Indel Realignment	No
Indel Repeat Filter Cutoff	None
Manifest	None
Manifest Padding	None
Picard HS Metric Reporting	None
Reverse Complement	None
Read Stitching	None
Variant Caller	GATK

Field	Configuration
Applicable analysis fields for the selected Workflow	• Aligner (BWA-Backtrack Legacy, BWA-MEM) • Export to gVCF • Flag PCR Duplicates • Genome Folder (fill in the folder path) • Indel Realignment • Variant Caller (Starling, GATK)

Library QC

Field	Configuration
Workflow	Library QC
Read 1 Cycles	251
Custom Primers	None
Aligner	None
Annotation	None
Export to gVCF	None
Flag PCR Duplicates	Yes
Genome Folder	Required
Indel Realignment	None
Indel Repeat Filter Cutoff	None
Manifest	None
Manifest Padding	None
Picard HS Metric Reporting	None
Reverse Complement	None
Read Stitching	None
Variant Caller	None
Applicable analysis fields for the selected Workflow	Flag PCR Duplicates Genome Folder (fill in the folder path)

GenerateFastQ

Field	Configuration
Workflow	GenerateFastQ
Read 1 Cycles	251
Custom Primers	None
Aligner	None
Annotation	None
Export to gVCF	None
Flag PCR Duplicates	None
Genome Folder	None
Indel Realignment	None
Indel Repeat Filter Cutoff	None
Manifest	None
Manifest Padding	None
Picard HS Metric Reporting	None
Reverse Complement	None
Read Stitching	None
Variant Caller	None
Applicable analysis fields for the selected Workflow	None

DNA Enrichment

Field	Configuration
Workflow	DNA Enrichment
Read 1 Cycles	251
Custom Primers	None
Aligner	BWA-MEM
Annotation	None
Export to gVCF	None
Flag PCR Duplicates	Yes

Field	Configuration
Genome Folder	Required
Indel Realignment	Yes
Indel Repeat Filter Cutoff	None
Manifest	Required
Manifest Padding	150
Picard HS Metric Reporting	No
Reverse Complement	None
Read Stitching	None
Variant Caller	Starling
Applicable analysis fields for the selected Workflow	• Aligner (BWA-Backtrack Legacy, BWA-MEM) • Flag PCR Duplicates • Genome Folder (fill in the folder path) • Indel Realignment • Indel Repeat Filter Cutoff (if Variant Caller is Somatic) • Manifest (fill in the file name) • Manifest Padding • Picard HS Metric Reporting • Variant Caller (Starling, Somatic, GATK) • Variant Frequency Percentage (if Variant Caller is Somatic)

DNA Amplicon

Field	Configuration
Workflow	DNA Amplicon
Read 1 Cycles	251
Custom Primers	None
Aligner	BWA
Annotation	RefSeq
Export to gVCF	None
Flag PCR Duplicates	None
Genome Folder	Required

Field	Configuration
Indel Realignment	Yes
Indel Repeat Filter Cutoff	None
Manifest	Required
Manifest Padding	None
Picard HS Metric Reporting	None
Reverse Complement	None
Read Stitching	None
Variant Caller	Germline
Variant Caller Depth Filter	10
Variant Quality Filter	30
Applicable analysis fields for the selected Workflow	• Aligner (BWA, TruSeq Amplicon Aligner) • Annotation (RefSeq, Ensembl) • Genome Folder (fill in the folder path) • Indel Realignment • Manifest (fill in the file name) • Read stitching (if Aligner is TruSeq Amplicon Aligner) • Variant Caller (Germline, Somatic) • Variant Caller Depth Filter (10–10000) • Variant Frequency Percentage (if Variant Caller is Somatic) • Variant Quality Filter (2–1000)

Global Fields

The following table shows the global fields that are configured to display on the Queue, Ice Bucket, and Record Details screens of the Denature and Dilute (MiSeq v3.2) step:

The Submitted Sample field, Progress, (added from previous MiSeq workflow) is obsolete in MiSeq v3.2.

Table 409 Global Field Configuration for Denature and Dilute (MiSeq v3.2) Step

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Configured on submitted sample:			
Read Length *Displays on Queue & Ice Bucket screens*	Text		Not applicable
Sequencing Method *Displays on Queue & Ice Bucket screens*	Text Dropdown	Custom Entries	Presets • Single Read • Paired End Read • Indexed Single Read • Indexed Paired End Read
Configured on derived sample:			
Final Loading Concentration *Displays on Record Details screen*	Numeric Dropdown	Required Field Custom Entries	Presets • 225 • 400 Decimal places displayed = 0

Step File Placeholders

Placeholders for the following files are configured on the Record Details screen of the Denature and Dilute (MiSeq v3.2) step:

- Lab Tracking Form (manually generated)
- MiSeq SampleSheet (autogenerated)
- MiSeq SampleSheet Generation Log (autogenerated)
- Log File (autogenerated)

Table 410 Sample Sheet Files and Placeholders Generated by Denature and Dilute (MiSeq v3.2) Step

Item	Description
Lab Tracking Form	This item in Clarity LIMS allows the lab scientist to attach a lab-specific tracking form to the step manually.

Item	Description
MiSeq SampleSheet	This CSV file is automatically generated by Clarity LIMS for use with the MiSeq system. The file can be opened as a text file or an Excel spreadsheet.
MiSeq SampleSheet Generation Log	This log file is automatically generated by Clarity LIMS. The log file captures any errors that Clarity LIMS might encounter when generating the sample sheet.
Log File	This log file is automatically generated by Clarity LIMS. The log file captures the status of the EvaluateDynamicExpression script that is invoked by the Set Next Step - Advance automation.

User Interaction

1. On the Queue screen, add the pool to the Ice Bucket, and then select View Ice Bucket.
2. On the Ice Bucket screen, select Begin Work.
3. On the Placement screen, do as follows.
   a. Scan the barcode of the MiSeq reagent cartridge into the MiSeq Reagent Cartridge field.
   b. Place the pool of samples into the reagent cartridge.
   c. Select Record Details.
4. On the Record Details screen, do as follows.
   a. Under Reagent Lot Tracking, select the reagent lot used in the step.
   If the reagent lot is not listed, add/activate the lot on the Reagents and Controls screen.
   b. Populate the fields in the Step Details table, as needed. Workflow, Experiment Name, and Read 1 Cycles are required fields. For details on how to populate the fields by using the preset drop-down list, refer to *MiSeq Integration v8.2 Validation and Troubleshooting* on page 2002.
   c. In the Sample Details table, enter the Final Loading Concentration. Select from the following preset options or enter a different value.
      - 225 (for PCR-free workflows)
      - 400 (for Nano workflows)
   d. Select Validate Run Setup and Generate MiSeq SampleSheet.
   Clarity LIMS generates the sample sheet and attaches it and a log file to placeholders in the Files area of the Record Details screen.
   e. Download the files and validate their format and content.

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5. Select Next Steps.
   On the Assign Next Steps screen, samples are already assigned to the MiSeq Run (MiSeq v3.2) step.
6. Select Finish Step.

Step 3: MiSeq Run (MiSeq v3.2)

Set Next Step - Advance Automation

This automation advances samples to the next step in the protocol. The automation is automatically triggered by exiting the Record Details screen.

The default command line is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-t false \
-h false \
-exp 'nextStep = ::ADVANCE::' \
-log {compoundOutputFileLuid5}"
```

Master Step Fields

The following fields are configured on the MiSeq Run (MiSeq v3.2) step. These fields display on the Record Details screen at run time. The read-only fields are automatically populated at the end of the run.

Table 411 MiSeq Run (MiSeq v3.2) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Drop-Down Items
Chemistry	Text	Read Only	Not applicable
Comment	Multiline Text		Not applicable
Experiment Name	Text	Read Only	Not applicable
Finish Date	Date	Read Only	Not applicable
Flow Cell ID	Text	Read Only	Not applicable
Flow Cell Version	Text	Read Only	Not applicable
Index 1 Read Cycles	Numeric	Read Only	Decimal Places Displayed: 0

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Field Name	Field Type	Options	Additional Options and Drop-Down Items
Index 2 Read Cycles	Numeric	Read Only	Decimal Places Displayed: 0
Output Folder	Text	Read Only	Not applicable
PR2 Bottle ID	Text	Read Only	Not applicable
Read 1 Cycles	Numeric	Read Only	Decimal Places Displayed: 0
Read 2 Cycles	Numeric	Read Only	Decimal Places Displayed: 0
Reagent Cartridge ID	Text	Read Only	Not applicable
Reagent Cartridge Part #	Text	Read Only	Not applicable
Run ID	Text	Read Only	Not applicable
Run Type	Text	Read Only	Not applicable
Status	Text	Read Only	Not applicable
Workflow	Text	Read Only	Not applicable

Global Fields

There are several sample and measurement global fields that are displayed on the Record Details screen of the MiSeq Run (MiSeq v3.2) step. These fields are autopopulated at the end of the sequencing run.

For more information, refer to *User Interaction* on page 1993 and *Sequencing Results Parsing* on page 1993.

Step File Placeholders

Placeholders for the following files are configured on the Record Details screen of the MiSeq Run (MiSeq v3.2) step:

- Illumina Run Report (autogenerated)
- Link to Run Folder (autogenerated)
- Run Parameters (autogenerated)
- Run Info (autogenerated)
- Lab Tracking Form (manually generated)

- Log File (autogenerated)

For details, refer to *Generated and Captured Files* on page 1994.

User Interaction

1. On the Queue screen, add the pool to the Ice Bucket, and then select View Ice Bucket.
2. On the Ice Bucket screen, select Begin Work.
3. On the Record Details screen, the fields in the table are read-only. After the run completes, the integration automatically performs the following actions:
   - Populates the fields.
   - Attaches files to the Illumina Run Report, Link to Run Folder, Run Parameters, and Run Info placeholders.
   - Populates the fields in the Sample Details table.
4. Select Next Steps.
   On the Assign Next Steps screen, the next step is already set to Mark protocol as complete.
5. Select Finish Step.

Sample Sheet Generation

Sample sheet generation occurs in the Denature & Dilute (MiSeq v3.2) step. This step places samples on the container loaded in the system.

The default configuration provides only the Validate Run Setup and Generate MiSeq SampleSheet automation. This automation uses the Template File Generator (`DriverFileGenerator.jar`) and a template file to generate a CSV format file for use with the MiSeq Control Software (MCS).

The sample sheet content is determined by the fields on the Record Details screen of the step in the Step Data table. The values entered into these fields are used to populate the sample sheet.

To customize the template used to create the sample sheet, you can insert additional columns.

The following additional details are available:

- For a sample template that you can download and customize for the lab, refer to *Illumina Instrument Sample Sheets (NGS v5.17 & later*.
- For instructions on customizing the template, refer to *Creating Template Files*.

Sequencing Results Parsing

The MiSeq Run (MiSeq v3.2) step records information for the flow cell lane and generates a report summarizing the results. In addition, run parameters, run info, and a link to the run folder are automatically captured.

Generated and Captured Files

The following table describes the run information files, reports, placeholders, and links that Clarity LIMS automatically generates or captures during a sequencing run:

Table 412 Run Information Generated or Captured by MiSeq Run (MiSeq v3.2) Step

Item	Description
Run Info Run Parameters	These XML files are automatically captured by Clarity LIMS from the run folder of the system. They include the key run parameters that are parsed out into step custom fields in Clarity LIMS.
Link to Run Folder	This link is the path to the network run folder where the data that was captured from the system during the run is stored. The link is automatically generated by Clarity LIMS.
Illumina Run Report	This report provides key information about the run and the samples on the flow cell. The report is automatically generated by Clarity LIMS. Information includes the flow cell ID, run directory location, and primary analysis metrics for the sequencing run. Information is summarized per flow cell lane for the entire run and individual reads for paired-end runs. These metrics are compared against the per lane averages of the sequencing run. The per lane averages are calculated using metrics from the last five sequencing runs. Any values outside of one standard deviation are highlighted.
Lab Tracking Form	This item in Clarity LIMS allows you to manually attach a lab-specific tracking form to the step.

Metadata

The following list shows metadata that Clarity LIMS automatically captures from the Illumina sequencing software as part of a sequencing run. This information is gathered from various run result files and events.

- Chemistry
- Experiment Name (entered in software)
- Finish Date (run completion date)
  - If the End Run event contains a date in the format YYYY-MM-DD, Finish Date is set to the date in the event file.
  - If the End Run event does not contain a date or the date is in the wrong format, Finish Date is set to the date when the event file is processed.

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- Flow Cell ID
- Flow Cell Version
- Index 1 Read Cycles (intended Index cycles)
- Index 2 Read Cycles (intended Index cycles)
- Output Folder (run folder root)
- PR2 Bottle ID
- Reagent Cartridge ID
- Reagent Cartridge Part #
- Read 1 Cycles
- Read 2 Cycles
- Run ID (the unique run ID)
- Run Type
- Status (current status of the sequencing run on the instrument)
- Workflow

Primary Analysis Metrics

The following table lists the real-time analysis (RTA) primary analysis metrics Clarity LIMS automatically captures and records per read, for samples in each flow cell lane. These metrics are captured upon run completion and are stored as fields in the Sample Details table on the Record Details screen.

To see both per read and per lane metrics, expand the output.

Table 413 RTA Primary Analysis Metrics Captured by MiSeq Run (MiSeq v3.2) Step

Per Read LIMS Field Name *(Stored on derived sample/analyte input to the step)*	Per Lane LIMS Field Name *(Stored in measurement placeholders in the Sample Details table on the Record Details screen)*
% Aligned R1	% Aligned R1
% Aligned R2	% Aligned R2
% Bases >=Q30 R1	% Bases >=Q30 R1
% Bases >=Q30 R2	% Bases >=Q30 R2
% Error Rate R1	% Error Rate R1
% Error Rate R2	% Error Rate R2
Not applicable	% Phasing R1

Per Read LIMS Field Name *(Stored on derived sample/analyte input to the step)*	Per Lane LIMS Field Name *(Stored in measurement placeholders in the Sample Details table on the Record Details screen)*
Not applicable	% Phasing R2
Not applicable	% Prephasing R1
Not applicable	% Prephasing R2
Not applicable	%PF R1
Not applicable	%PF R2
Not applicable	Cluster Density (K/mm^2) R1
Not applicable	Cluster Density (K/mm^2) R2
Intensity Cycle 1 R1	Intensity Cycle 1 R1
Intensity Cycle 1 R2	Intensity Cycle 1 R2
Not applicable	Reads PF (M) R1
Not applicable	Reads PF (M) R2
Yield PF (Gb) R1	Yield PF (Gb) R1
Yield PF (Gb) R2	Yield PF (Gb) R2

How It Works

1. The sequencing service runs on the Clarity LIMS server. The service detects event files that instrument RTA produces as the run progresses. The event files let the service know where to find the run data.
2. As the run data are written out and the End Run event is detected, the following events occur:
   - The data are matched to the step based on the reagent cartridge ID that was entered or scanned on the Denature and Dilute (MiSeq v3.2) step.
   - Read-only field values on the Record Details screen are populated accordingly.
3. When the service has finished processing the end run event and updating the fields in Clarity LIMS, the sequencing service generates the report and attaches it to the step.

User Interaction

1. Manually start the step. Leave the step in progress until the sequencing service has finished processing the run and the run report is generated and attached.
2. After the run has completed, verify the following:
   - The read-only fields on the Record Details screen are populated.
   - The Illumina Run Report is generated and attached.

- The Link to Run Folder is generated and attached.
- The Run Parameters and Run Info files are attached.

Leave the step as in progress until the Illumina Run Report has been attached to the step in Clarity LIMS.

3. [Optional] In the Files section, upload the Lab Tracking Form.
4. Select Next Steps.
   On the Assign Next Steps screen, the next step is already set to Mark protocol as complete. The Log File is attached when the next step for samples has been assigned by the Next Step - Advance automation.
5. Select Finish Step.

Scripts and Files Installed

This integration requires components installed with the Illumina Preset Protocols (IPP).

The Illumina MiSeq Integration Package v8.2 RPM installs the scripts and files listed in the following table.

Component	Location & Description
`configure_extensions_miseq_sequencingservice-v8.sh`	`/opt/gls/clarity/config/` Script that installs the service properties in the database.
`log4j.xml`	`/opt/gls/clarity/extensions/Illumina_MiSeq/v8/SequencingService/conf` File containing the settings for logging the sequencing jar.
`miseq-sequencing-v8.jar`	`/opt/gls/clarity/extensions/Illumina_MiSeq/v8/SequencingService` Jar file containing API-based Clarity LIMS extensions used for capturing run results and report generation.
InterOp libraries	`/opt/gls/clarity/extensions/Illumina_MiSeq/v8/lib` Illumina shared library for parsing InterOp data files.
`MiSeqSamplesheetv3.csv` (Installed by IPP)	`/opt/gls/clarity/extensions/conf/driverfiletemplates` Template file used for file generation.

Database Properties Installed

The database properties installed in the Illumina MiSeq Integration v8.2 are described in the following section.

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The integration also installs several additional database properties, each with the 99 suffix appended to their name, that are not listed in the table. These properties are intended for use by the Clarity LIMS support team in automated validation tests.

Sequencing runs are matched using the reagent cartridge ID and the base name of the sequencing step, MiSeq Run (MiSeq v3.2).

Do not change the base name. The sequencing service that captures sequencing run results expects this name. The base name is stored in the `sequenceProcessBaseName` database property. If the name is changed without the database property being updated, the reagent cartridge ID <-> sequencing step base name matching system fails.

If you need to rename the step, for example to add a version number, modify the text before or after the base name portion. This part of the name is not used in the matching system. For example, change MiSeq Run (MiSeq v3.2) to Demo-MiSeq Run (MiSeq v3.2)-Updated.

Table 414 Database Properties Installed with the Illumina MiSeq Integration Package

Property	Description	Default Value
`miseq.v8.seqservice.sequenceProcessBaseName`	Sequencing process type/master step base display name. Partial matching is used to look up the process type/master step. This property is added/updated by Illumina Preset Protocols (IPP) during the workflow installation.	MiSeq Run (MiSeq v3.2)
`miseq.v8.seqservice.eventFileDirectory.1`	A network location monitored for event files, eg, `/mnt/illumina/gls_events/`	/mnt/gls_events

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Property	Description	Default Value
`miseq.v8.seqservice.netPathPrefixSearch.1`	The network directory prefix contained in the event file.	\\nas\network\run_data
`miseq.v8.seqservice.netPathPrefixReplace.1`	The mapped network directory mount name on the server used to access the run data directory, eg, `/mnt/network/data`	/mnt/run_data
`miseq.v8.seqservice.eventFileDirectorySuffixes`	A list of eventFileDirectory path entries to monitor for event files. The value is one or more comma-separated integers.	99 Configured on install to point to 1
`miseq.v8.seqservice.netPathPrefixSearchReplaceSuffixes`	A list of netPathPrefix search and replace entries for transforming Windows to Linux network paths. The value is one or more comma-separated integers.	99 Configured on install to point to 1

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Property	Description	Default Value
`miseq.v8.seqservice.runReportViewsVersion`	The current version of the Run Report views in the database. The value 0 represents the state before the views are created. This property is automatically updated by the run report.	0
`miseq.v8.seqservice.ignoreUnmatchedContainerIds`	A flag indicating if event files that cannot be matched to reagent cartridges in Clarity LIMS should be archived after a certain period (true) or continually reprocessed (false).	false To prevent the gls_events file directory from becoming cluttered, it is recommended that the value of this property is set to true
`miseq.v8.seqservice.ignoreUnmatchedContainerIdsWaitDays`	The number of days between when the event is created and the event file is archived.	14
`miseq.v8.seqservice.synchronizationPeriod`	Invocation period in seconds.	60

You can configure support for multiple identical seqservice.netPathPrefixSearch property values. For more information, refer to *Configure Multiple Identical netPathPrefixSearch Values* on page 2596.

Consumables Installed

Reagent Categories and Kits

Reagent categories or label groups are installed with the IPP workflow configuration slices.

The MiSeq Reagent Kit is included in the Illumina MiSeq Integration.

Control Types

The PhiX v3 control type is included in the Illumina MiSeq Integration.

Container Types

The following container types are included in the Illumina MiSeq Integration:

- MiSeq Reagent Cartridge
- 96 well plate
- Tube

All one-dimensional container types with both numeric rows and numeric columns are supported.

Instrument Integration

To make sure that the Illumina instrument warranty remains valid, the instrument integration must be performed and maintained by the Clarity LIMS Support team. To perform this integration, the Support team requires remote access to the system while it is idle.

The following steps are performed by the Clarity LIMS Support team when configuring the sequencing for use with the Illumina MiSeq Integration.

1. Create a directory on the local computer to hold the batch files. These batch files write event files to the network attached storage (NAS) shares.
2. Create a directory on the NAS to hold the event files.
3. Modify Illumina software configuration files to call the batch files that create the event files.
4. Update sequencing service default database properties to match the specifics of the installation.

Rules and Constraints

The Illumina MiSeq Integration operates with the following constraints:

- The reagent cartridge ID must be unique. There should not be multiple reagent cartridge containers in the system with the same name.
- The reagent cartridge ID must be scanned as the reagent cartridge Container Name on the Denature & Dilute (MiSeq v3.2) step.

MiSeq Integration v8.2 Validation and Troubleshooting

The following sections explain how to validate the installation of the Illumina MiSeq Integration Package v8.2.

The validation involves the following processes:

- Running samples through the Library Prep Validation v2.3.1 workflow. The workflow contains a single-step protocol that models the library prep required to produce normalized libraries. At the end of the step, the normalized libraries automatically advance to the MiSeq Sequencing v3.2 workflow.
- Running normalized libraries through the MiSeq Sequencing v3.2 workflow. This process validates the following information:
  - Automated generation of a sample sheet for use with the MiSeq Control Software (MCS) and Local Run Manager (LRM).
  - Automated tracking of the MiSeq sequencing run and parsing of run statistics into Clarity LIMS, including the following information:
    - Run status and metrics of sequencing run
    - Sequencing run parameters
    - Real-Time Analysis (RTA) v1 run directory location and other run-specific information

Activate Workflow, Create Project, Add and Assign Samples

The following steps prepare Clarity LIMS to run samples through the Library Prep Validation and MiSeq Sequencing v3.2 workflows.

1. In the Clarity LIMS Configuration area, select each workflow and change the Workflow Status toggle to Active.
2. On the Projects & Samples screen, create a project. Add samples to the project.
3. Assign the samples to the Library Prep Validation workflow.

Library Prep Validation Protocol

This single-step protocol models the library prep required to produce normalized libraries that are ready for the MiSeq Sequencing v3.2 workflow.

Run Library Prep Validation Step

1. In Lab View, locate the Library Prep Validation protocol. Samples are queued for the Library Prep Validation step. Select the step to proceed to the Queue screen.
2. On the Queue screen, add the samples to the Ice Bucket, and then select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Placement screen, do as follows.

a. In the container field in the Placed Samples area, scan or enter the barcode of the destination container.
b. Select samples from the Samples to be Placed area and drag them to the container wells on the right. Repeat until all samples are placed.
c. Select Add Labels.

5. On the Add Labels screen, do as follows.
   a. To assign labels, select a label and drag it to a container well. Repeat as needed.
   b. Select Record Details.
6. On the Record Details screen, do as follows.
   a. Select all the samples in the Sample Details table.
   b. In the Sequencing Instrument drop-down list, select MiSeq.
   c. Select Apply to set this value for all selected samples.
   d. Select Next Steps.
   On the Assign Next Steps screen, the next step is automatically set to Remove from Workflow. This setting is required by the Routing script automation.
7. Select Finish Step.
   The Routing script automation is triggered. Samples are routed to the first step in the MiSeq Sequencing v3.2 protocol—Library Pooling (MiSeq v3.2).

MiSeq Sequencing v3.2 Protocol

Run Library Pooling (MiSeq v3.2)

1. In Lab View, locate the MiSeq Sequencing v3.2 protocol. Samples are queued for the Library Pooling (MiSeq v3.2) step. Select the step to proceed to the Queue screen.
2. On the Queue screen, do as follows.
   a. Add the samples to the Ice Bucket.
   b. In the Add Control Samples panel, add the PhiX v3 control sample to the Ice Bucket.
   c. Select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
   The Validate Maximum Number of Samples automation script runs. The maximum number of samples allowed is 1536.
4. On the Pool Samples screen, do as follows.
   a. Create a pool of samples by dragging samples into the Pool Creator.
   b. Name the pool or accept the default name (Pool #1).
   c. Select Place Samples.
5. On the Placement screen, do as follows.

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a. Select the pool from the Samples to be Placed area and drag it to the container.
b. Select Record Details.

6. On the Record Details screen, select Next Steps.
   On the Assign Next Steps screen, next step is automatically set to Denature and Dilute (MiSeq v3.2).
7. Select Finish Step.

Run Denature and Dilute (MiSeq v3.2)

1. In Lab View, locate the MiSeq Sequencing v3.2 protocol. The pooled samples are queued for the Denature and Dilute (MiSeq v3.2) step. Select the step to proceed to the Queue screen.
2. On the Queue screen, add the pool to the Ice Bucket, and then select View Ice Bucket.
   Only one pool is allowed as an input.
3. On the Ice Bucket screen, select Begin Work.
   The Validate Single Input automation script runs.
4. On the Placement screen, do as follows.
   a. Scan the MiSeq reagent cartridge barcode into the MiSeq Reagent Cartridge field.
   b. Place the pool of samples into the reagent cartridge.
   c. Select Record Details.
5. On the Record Details screen, do as follows.
   a. In Reagent Lot Tracking, select the reagent lot used in the step.
      If the reagent lot is not listed, add/activate the lot on the Reagents and Controls screen.
   b. Use the Preset drop-down list to help populate the fields in Step Details, as needed. For more information on presets, refer to *Using Step Details Presets* on page 2005. Workflow, Experiment Name, and Read 1 Cycles are required fields.
   c. In the Sample Details table, enter the Final Loading Concentration. Select from the following preset options or enter a different value.
      - 225 (for PCR-free workflows)
      - 400 (for Nano workflows)
   d. Select Validate Run Setup and Generate MiSeq SampleSheet.
      Clarity LIMS generates the sample sheet and attaches it and a log file to placeholders in the Files area of the Record Details screen.
   e. Download the files and validate the format and content.
6. Select Next Steps.
   On the Assign Next Steps screen, samples are automatically assigned to the MiSeq Run (MiSeq v3.2) step.
7. Select Finish Step.

Using Step Details Presets

Most of the custom fields in Step Details are analysis settings used by Local Run Manager (LRM) and are for advanced users only. The following fields are exceptions:

- Workflow
- Experiment Name
- Description
- Read 1 Cycles
- Read 2 Cycles
- Custom Primers

To use the default analysis settings, select the analysis module from the Preset drop-down list. The default values populate the respective fields and indicate the following actions are needed:

- Required—The field is required for the specific analysis module selected. Replace the word Required with a proper value. For example, the Genome Folder field should contain the directory to the genome folder.
- None—The field is not applicable for the specific analysis module selected. Leave the field as is.

For a list of fields that are applicable for the analysis module selected, refer to the Applicable analysis fields for the selected Workflow field in Step Details.

Run MiSeq Run (MiSeq v3.2)

1. In Lab View, locate the MiSeq Sequencing v3.2 protocol. The pool of samples is queued for the MiSeq Run (MiSeq v3.2) step. Select the step to proceed to the Queue screen.
2. On the Queue screen, add the pool to the Ice Bucket, and then select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Record Details screen, the fields are read-only. Select the applicable instrument from the Instrument Used dropdown and select Save.
   For more information on adding instruments, refer to *Add and Configure Instruments* in the *Clarity LIMS Core Functionality* documentation.
5. When the run completes, the integration automatically performs the following actions:
   - Populates the fields.
   - Attaches files to the Illumina Run Report, Link to Run Folder, Run Parameters, and Run Info placeholders.
   - Populates the fields in the Sample Details table. For details, refer to *MiSeq Integration v8.2 Configuration and User Interaction* on page 1969.

   The Log File is attached after the next step for samples is assigned by the Next Step - Advance automation. Make sure the Illumina Run Report has been attached before continuing.

6. Select Next Steps.
   On the Assign Next Steps screen, the next step is automatically set to Mark protocol as complete.
7. Select Finish Step.

Validating Creation of Event Files

This validation checks the following information:

- The destination path is correctly configured.
- The instrument computer can access and write to the destination path.
- There are no syntax errors in the Clarity LIMS batch file.

Use the following steps to confirm that event files are created by the batch file in the destination path. The steps assume that the default configuration has been successfully imported.

1. In `C:\Illumina\gls`, double-click the batch file `gls_event_mcs_rta_lrm.bat`.
2. Confirm that an empty event file appears in the configured DESTINATION_PATH.

Manual execution of the batch file produces an output resembling the following example:

```
Filename:
event-EndRun-07295667.txt
Contents:
cycleNumber =
runFolder =
netFolder =
readType =
eventType = EndRun
softwareType = MCS
finishDate = 2021-01-25
```

Troubleshooting

If an automation trigger does not appear to run the corresponding scripts, see the following topics:

- *Troubleshooting Automation Worker* in the *Clarity LIMS Core Functionality* documentation.
- *Troubleshooting Automation* in the *Clarity LIMS API & Database* documentation.

If an error occurs that does not provide direction on how to proceed, confirm the version of the installed MiSeq Integration Package. To confirm the version, run the `rpm -qa | grep -i miseq` command from the server console.

If the error is related to data parsing, retrieving run results data, or report values not appearing as expected, review the `MiseqIntegrator.log` file. The file is located at `/opt/gls/clarity/extensions/Illumina_MiSeq/v8/SequencingService`.

Additional troubleshooting information for this integration is provided in the *Illumina Instrument Integrations FAQ*.

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If you are unable to resolve the issue, contact the Clarity LIMS support team. Supply the relevant information from the troubleshooting already performed.

MiSeq Integration v8.2 Installation

The Illumina MiSeq Integration Package v8.2 supports the integration of Clarity LIMS to MiSeq instruments.

Integration Overview

MiSeq Integration v8.2 includes the following components:

- Preconfigured MiSeq Sequencing v3.2 workflow that maps to lab protocol steps and instrument runs.
- Automated sample sheet generation for use with MiSeq Control Software (MCS) and Local Run Manager.
- The following supported Local Run Manager analysis modules:
  - Generate FASTQ
  - Library QC
  - Resequencing
  - DNA Enrichment
  - DNA Amplicon
- Automated tracking of the sequencing run and the parsing of the following run statistics to Clarity LIMS:
  - Sequencing run metrics
  - Sequencing run parameters
  - Real-Time Analysis RTA v1 run directory location (and other run specific information)
- Preconfigured Library Prep Validation workflow that is used for validation purposes only. This workflow contains a single step protocol that models the library prep required to produce normalized libraries that are ready for the MiSeq Sequencing v3.2 workflow. MiSeq Integration Package v8.2 works with Library Prep Validation v2.3.1, which is installed with Illumina Preset Protocols (IPP) v2.6.

MiSeq Integration v8.2 runs as independent service and can be on the same server as other Clarity LIMS integrations. The integration is only required for MCS v4.0. If you are running MCS v3.0 or below, do not upgrade to this integration.

Compatibility

MiSeq Integration v8.2 is compatible with the following software:

- Clarity LIMS v6.2 and later

- Secret Util v1.0 and later
- IPP v2.6 and later
- MCS v4.0/LRM v3.0 and later
- Oracle Linux (for compatibility, refer to *MiSeq Integration v8.2 Release Notes* on page 2018)

Prerequisites

MiSeq Integration v8.2 has the following prerequisites:

- Mount run data network-attached storage (NAS) share
- IPP is installed

Prerequisite 1: Mount Run Data NAS Share

Mounting the NAS share of run data are needed to capture and generate files associated with the sequencing run. To mount NAS shares that contain data from the Clarity LIMS server, use Read/Write privileges as the glsjboss user. The following data can be mounted to the NAS share:

- Run data (eg, `\\network-storage\run_data\`)
- Clarity LIMS-created events triggered by the End Run event of the Illumina sequencing run (eg, `\\network-storage\illumina\gls_events\`)

With Read access, the Clarity LIMS server reads the following information in individual sequencing run data folders:

- Run information metadata from `<runFolderRoot>/RunInfo.xml and <runFolderRoot>/runParameters.xml`.
- Run statistics from `<runFolderRoot>/InterOp/*.bin`.

The Clarity LIMS server generates the following files and information locally and stores them in Clarity LIMS:

- Sample sheet (CSV file)
- Run report (PDF file)
- Run folder root link

The Clarity LIMS server copies and stores the following files from individual sequencing run data folders in Clarity LIMS:

- `<runFolderRoot>/RunInfo.xml`
- `<runFolderRoot>/runParameters.xml`

Prerequisite 2: IPP Installation

MiSeq Integration Package v8.2 depends on the QC Protocols configuration provided in IPP (v2.6 and later) for Clarity LIMS v6.2 and later.

If the base configuration is not installed, then install it on the Clarity LIMS server that is being used for the MiSeq integration. For details on IPP v2.6 installation and configuration, refer to the *Illumina Preset Protocols* documentation.

If you are upgrading the base configuration, make sure that the IPP package is compatible with the version of Clarity LIMS you are installing (in this case, IPP v2.6 for Clarity LIMS v6.2).

If you do not have QC Protocols, then install them as follows.

1. As a glsjboss user, run the following command to view the complete list of IPP workflows:

```
/opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o list
```

2. To install the dependent QC Protocols, run the following command:

```
/opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o install QC_
Protocols.qc-protocols
```

Installation

MiSeq Integration v8.2 is distributed as the ClarityLIMS-Illumina-MiSeq-Package-v8 RPM package that must be installed on the Clarity LIMS server. This package installs the following items:

- Bash scripts used to run the service
- The `miseq-sequencing.jar` file
- The `configure_extensions_miseq_sequencingservice.sh` script
- Smoke test directories

The following installation steps are required for the installation of MiSeq Integration v8.2.

The MiSeq RPM must be installed on the Clarity LIMS server. This is the same location where the AI node or Automation Worker is installed. The automations and sequencing service use the existing Automation Worker.

Install the RPM

1. On the Clarity LIMS server, log in as the root user.
2. Run the following yum command to install the RPM:

   You must use the `--enablerepo` command line argument to enable the repo. For the repo file and the correct name to use, contact Illumina Support.

```
yum install ClarityLIMS-Illumina-MiSeq-Package-v8 --enablerepo=< repo name info
from support >
```

3. Enter `y` to confirm that you want to proceed with the RPM installation.
   After confirmation, you are prompted to install the MiSeq workflow from the IPP script.
4. Install the MiSeq workflow as follows.

   > The following steps assume that the QC protocols from the IPP package have been installed. For more information, refer to *Prerequisite 2: IPP Installation* on page 2009.

   a. As the glsjboss user, run the following command to view the complete list of IPP workflows:

   ```
   /opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o list
   ```

   b. Run the following command to install the MiSeq Sequencing v3.2 workflow configuration:

   ```
   /opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o install
   Illumina_Instruments.miseq-v3.2
   ```

   The installer does the following tasks:

   - Validates the import of the workflow and provides the Warning/Error details in STDOUT, which allows you proceed with the import or cancel it.
   - Automatically detects the version of Clarity LIMS and imports the applicable workflow configuration.
   - Creates or updates the miseq.v8.seqservice.sequenceProcessBaseName database property, which can be verified with the omxProps-ConfigTool utility. This property shows the sequencing master step base display name. It can also use partial matching to look up the master step by the sequencing service. The default value is MiSeq Run (MiSeq v3.2).

   c. [Optional] Run the following command to install the Library Prep Validation v2.3.1 workflow that is used to validate the MiSeq Sequencing v3.2 workflow:

   ```
   /opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o install
   Illumina_Instruments.Library-Prep-Validation-v2.3
   ```

   The installer does the following tasks:

   - Validates the import of the workflow and provides the Warning/Error details in STDOUT, which allows you proceed with the import or cancel it.
   - Automatically detects the version of Clarity LIMS and imports the applicable workflow configuration.

5. Run the following script to configure the service properties:

```
/opt/gls/clarity/config/configure_extensions_miseq_sequencingservice-v8.sh
```

The script creates the configuration properties in the database. For a list of the configured properties, refer to Configure the Database Service Properties.

Configure the Database Service Properties

To enable capture and generation of files associated with the sequencing run, configure the following properties using the omxProps-ConfigTool utility.

Table 415 Configurable Database Properties

Property	Description	Default Value
miseq.v8.seqservice.eventFileDirectory.1	A monitored network location used for event files.	`/mnt/gls_events`
miseq.v8.seqservice.eventFileDirectory.99	The monitored smoke test location used for event files. This location is also used for the installation and upgrade of smoke tests.	`/opt/gls/clarity/smoketests/miseq/monitored`
miseq.v8.seqservice.netPathPrefixSearch.1	The network directory prefix contained in the event file.	`\\nas\network\run_data`
miseq.v8.seqservice.netPathPrefixSearch.99	The default benign smoke test search entry. This property is used for the installation and upgrade of smoke tests.	`/`

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Property	Description	Default Value
miseq.v8.seqservice.netPathPrefixReplace.1	The mapped network directory mount name on the server used to access the run data directory.	`/mnt/run_data`
miseq.v8.seqservice.netPathPrefixReplace.99	The default benign smoke test replace entry. This property is used for the installation and upgrade of smoke tests.	`/`
miseq.v8.seqservice.eventFileDirectorySuffixes	A list of eventFileDirectory path entries used to monitor for event files. The value is one or more comma separated integers.	99 When installed, this property must be configured to point to 1 unless a validation test is being performed with the 99 version of the search, replace, and event file entries.

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Property	Description	Default Value
miseq.v8.seqservice.netPathPrefixSearchReplaceSuffixes	A list of netPathPrefix search and replace entries for transforming Windows to Linux network paths. The value is one or more comma separated integers.	99 When installed, this property must be configured to point to 1 unless a validation test is being performed with the 99 version of the search, replace, and event file entries.

Start the Sequencing Service

1. Run the following command to start the sequencing service:

```
systemctl start miseq_seqservice-v8
```

Workflows, Protocols, and Steps Installed

MiSeq Integration v8.2 works with the MiSeq Sequencing v3.2, which contains a single protocol with the same name. This protocol includes the following steps:

- Library Pooling (MiSeq v3.2)
- Denature and Dilute (MiSeq v3.2)
- MiSeq Run (MiSeq v3.2)

For descriptions of the protocol and the steps, refer to *MiSeq Integration v8.2 Configuration and User Interaction* on page 1969. For instructions on using the Library Prep Validation v2.3.1 workflow to validate the automated sample sheet generation, refer to *MiSeq Integration v8.2 Validation and Troubleshooting* on page 2002.

Instrument Software

The MCS is divided into the following modules:

- MCS—Controls the instrument operation, including various configuration settings. This software is installed and runs on the instrument.

- Local Run Manager—Analyzes sequencing data and integrates with MCS. This software can be accessed on the instrument through a web browser.
- Real-Time Analysis (RTA)—Performs image processing and base calling (primary analysis). The software makes sure that data files are created and copied to the final destination folder and is installed and runs on the instrument.

For more information on MCS, refer to the MiSeq documentation at support.illumina.com.

Instrument Integration

The instrument integration must be performed and maintained by Illumina Support. Illumina Support requires remote access to the instrument while it is idle.

The support team has created batch files that use custom scripts during the key events of a sequencing run. When these batch files are used, they read the event information and write it in a TXT event file at the same network share location that the instrument uses to write the run data. Another process running on the Clarity LIMS server receives the event files and takes the appropriate actions.

The sequencing service monitors the end of the run event. This event is used to capture key step data and files and parse run statistics for output custom fields.

Configure Batch Files

When the instrument is running, the final destination for the run data are a network storage path. The software is configured with a network storage path root (eg, `\\network-storage\illumina\`). Each sequencing run generates a unique run ID, which is appended to create a unique data run directory (eg, `\\network-storage\illumina\110419_InstrumentName_0001_ARG1234567`).

The Clarity LIMS batch files must be configured to write to a directory within the network storage path root. This directory is typically named `gls_events`, but the directory name can be different as long as no spaces are used. The batch file used for MCS v4.0 is `gls_event_mcs_rta_lrm.bat`.

Before configuring the batch files, do the following:

1. Back up the `MiSeq.Configuration.xml` configuration file to `C:\Illumina\RTA\Configs\`.
2. Make sure that the instrument is idle.
3. Shut down MCS.
4. Set up the directory structure as follows.
    a. Create a directory (`C:\Illumina\gls` is recommended) on the local PC to hold the batch file. For Windows 10, the folder must be under `C:\Illumina` instead of `C:\Illumina\gls` because of Windows software restriction policies. If the folder is not in that directory, the batch script does not run. For versions before Windows 10, `C:\Illumina\gls` is acceptable.
    b. Create a directory (eg, `gls_events`) on the NAS to hold the event files.

Configure the batch files as follows.

1. Determine the site or instrument specific network storage path root.

2. Make the following changes to the `gls_event_mcs_rta_lrm.bat` batch file:

   Make sure to include a trailing \ in the DESTINATION_PATH and DEFAULT_LOCAL_OUTPUT_PATH lines. Refer to the following examples:

   ```
   set DESTINATION_PATH=\\network-storage\illumina\gls_events\
   set DEFAULT_LOCAL_OUTPUT_PATH=D:\Illumina\MiSeqTemp\
   ```

   a. Change the DESTINATION_PATH line to the name of the event file directory.

   b. Change the DEFAULT_LOCAL_OUTPUT_PATH line to the name of the default local temp run folder directory.
   The default directory is `D:\Illumina\MiSeqTemp\`.

3. Copy the DESTINATION_PATH and paste it into the Windows Explorer address bar.
4. Make sure that the network location is accessible and that it opens from the instrument.

The batch file must be deployed to the MiSeq instrument. This file must be deployed to its own directory (`C:\Illumina\gls\` is recommended) on the instrument computer so that it is not overwritten or removed during an instrument software update.

Deploy the batch file as follows.

1. From the Attachment section, download the batch file.
2. Copy the file to the directory that you created on the instrument computer (eg, `C:\Illumina\gls\`).
3. From the Command Prompt, run the following command to list the contents of the directory:

```
dir C:\Illumina\gls\
```

4. Make sure that the name of the batch file does not contain any special or hidden characters.

Connect Batch Files to Instrument Events

Update the MCS configuration files as follows.

1. Reboot the instrument.
2. Make a backup copy of the RTA configuration XML file and name it `MiSeq.Configuration.xml`.
3. Edit the file to connect to the RTA End Run event as follows.

   a. Open the `MiSeq.Configuration.xml` file at `C:\Illumina\RTA\Configs\`.

   b. Update the following content within the <ProcessCompleteEventFile> tags:

   ```
   <ProcessCompleteEventFile>C:\Illumina\gls\gls_event_mcs_rta_
   lrm.bat</ProcessCompleteEventFile>
   ```

4. Save the edited file.
5. Validate the file as follows.

   a. From `C:\Illumina\RTA`, create a backup of the `RTA.exe.config` file and name it `Backup_RTA.exe.config`.

   b. Open `RTA.exe.config` and change the DefaultConfigFile value to MiSeq.Configuration.xml.

c. Save the changes and close the file.

d. From the Command Prompt, run the following command:

```
C:\Illumina\RTA\RTA.exe "." processedfolder="."
```

e. If the validation is successful, a warning message displays that says the output path cannot be placed into the run directory. Select OK.

f. In the Command Prompt, make sure the following error message displays:

```
RunInfo.xml does not exist: System.ApplicationException: Error in the
application.,0,0
```

g. Press Ctrl + C to terminate the `RTA.exe` application that is still running.
If the configuration file has an error, the command returns specifics about the problem. Refer to the following example:

```
While loading RTA Configuration File
"C:\Illumina\RTA\Configs\MiSeq.Configuration.xml" --->
System.InvalidOperationException:
```

```
There is an error in XML document <39, 3>. ---> System.Xml.XmlException: The
'GLS' start tag on line 33 position 4 does not match the end tag of
'RTAConfiguration'. Line 39, position 3.
```

```
Error loading xml file: Unknown node 'GLS' at line 83 pos 5
```

6. After the validation is complete, go back to `C:\Illumina\RTA` and delete the current `RTA.exe.config` file.

   Make sure that you rename the backup file. If you do not rename the file, it can cause the RTA not to work properly.

7. Restore the backup file from a by renaming it `RTA.exe.config`.
8. Start MCS.
9. Open the `MiSeq.Configuration.xml` file and make sure that the changes were saved.

Validation

Sample Sheet Generation

For instructions on how to validate the automated sample sheet generation, refer to *MiSeq Integration v8.2 Validation and Troubleshooting* on page 2002.

Manual Invocation of Event Files

The manual invocation of an event file produces an output that contains the following information:

- File name (eg, `event-EndRun-07295667.txt`)
- cycleNumber (eg, 318)
- runFolder (eg, `"D:\Illumina\MiSeqTemp\230815_M99999_0028_FC1234567-ABCDE"`)
- netFolder (eg, `\\network-folder\Run_Data\230815_M99999_0028_FC1234567-ABCDE`)

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- readType (eg, 4)
- eventType (eg, EndRun)
- softwareType (eg, MCS)
- finishDate (eg, 2023-08-15)

If an event file is triggered automatically during a MiSeq run, then the completion event does not appear until the run has completed and RTA completes primary analysis.

For instructions on validating the creation of event files, refer to *MiSeq Integration v8.2 Validation and Troubleshooting* on page 2002.

Instrument Sequencing Run

The instrument sequencing run test validates that the Clarity LIMS batch file is connected properly and invoked on the instrument events. Before validating the batch file, make sure that the following prerequisites are met:

- You have access to the NAS share.
- The default configuration has been successfully imported.
- Manual invocation of the event files has been validated. This validation checks for the following information:
  - The DESTINATION_PATH and DEFAULT_LOCAL_OUTPUT_PATH are configured correctly.
  - The instrument computer can access and write to the DESTINATION_PATH.
  - There are no syntax errors in the Clarity LIMS batch script.

For more information on event file validation, refer to *MiSeq Integration v8.2 Validation and Troubleshooting* on page 2002.

The sequencing service processes and archives event files, which can cause validation issues while the service is running. You can make the following changes to avoid losing the event files that you are attempting to validate:

- Modify the FINAL_EXTENSION value in the Clarity LIMS batch file so that the file extension is .test instead of .txt. The service only processes and archives TXT files. Make sure that you change FINAL_EXTENSION back to .txt after manual validation.
- Monitor the `MiSeqIntegrator.log` file, which logs the file name and contents of each event file that is processed. The log file is at `/opt/gls/clarity/extensions/Illumina_MiSeq/v8/SequencingService`.

Validate the sequencing run as follows.

1. During the run, monitor the contents of the gls_events directory.
2. After the run is completed and the RTA completes primary analysis, make sure that a final EndRun event displays (eg, `event-EndRun-11043279.txt`).

MiSeq Integration v8.2 Manual Upgrade

MiSeq Integration v8.2 includes the MiSeq v3.2 workflow upgrade. This upgrade is typically done through the API. To upgrade the workflow manually, create the Validate Single Input automation and update its trigger information as follows.

Before updating the script, make sure the MiSeq v3.1 workflow is installed.

Create Validate Single Input Automation

1. In Clarity LIMS, under Configuration, select the Automation tab.
2. Select New Automation.
3. Create an automation called Validate Single Input.
4. In the Command Line field, add the following string:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:validateSampleCount -min 1 -max 1"
```

5. Under Automation Use, enable this automation on the Denature and Dilute master step.

Change Trigger Point for the Validate Single Input Automation

1. In Clarity LIMS, under Configuration, select the Lab Work tab.
2. Find the Denature and Dilute step.
3. In the Automation section, update the Trigger Location and Trigger Style for the Validate Single Input automation as follows.
   - Trigger Location—Step
   - Trigger Style—Automatic upon entry

MiSeq Integration v8.2 Release Notes

These Release Notes describe the key changes to software components for the Clarity LIMS MiSeq Integration Package since version 8.1.

Compatibility

This version of the integration package is compatible with the following software:

- Clarity LIMS v6.2 and later
- NGS Extensions Package v5.23 and later
- MiSeq Control Software (MCS) v4.0
- Local Run Manager v3.0
- Oracle Linux (64-bit) v8.8

New Features

- Upgraded Java and third-party dependency libraries.
- Upgraded Groovy to v3.0.7.

Defects Fixed

- The Denature and Dilute step now only allows a single pool to be added to the Ice Bucket when you enter the step.
- Fixed the MiSeq integration service not starting automatically after a system reboot.
- Fixed the SQL Injection (CVE-2022-31197) and Information Disclosure (CVE-2020-15522) vulnerabilities.

Known Issues

None

MiSeq Integration v8.1 Configuration and User Interaction

The Illumina MiSeq Integration Package v8.1 supports the integration of Clarity LIMS to Illumina MiSeq Sequencing Systems.

The integration allows for automated tracking of an Illumina sequencing run in Clarity LIMS. This capability includes tracking sequencing run status, generating run report, and capturing and parsing run statistics. In addition, this integration provides automated generation of a sample sheet file for use with the MiSeq Control Software (MCS) and Clarity LIMS Product Analytics.

Overview

The Illumina MiSeq Integration Package v8.1 includes the following features:

- Preconfigured MiSeq Sequencing v3.1 workflow that maps to lab protocol steps and sequencing runs.
- Automated generation of a sample sheet for use with the MiSeq Control Software (MCS) and Clarity LIMS Product Analytics.
- Supported Clarity LIMS Product Analytics analysis modules are GenerateFastQ, Library QC, Resequencing, DNA Enrichment, and DNA Amplicon.
- Automated tracking of the MiSeq sequencing run and parsing of run statistics into Clarity LIMS, including:
  - Run status and metrics of sequencing run
  - Sequencing run parameters
  - Real-Time Analysis (RTA) v1 run directory location and other run-specific information

This integration also includes a preconfigured Library Prep Validation workflow that is used for validation purposes only. The workflow contains a single-step protocol that models the library prep required to produce normalized libraries that are ready for the MiSeq Sequencing v3.1 workflow. For details, see *MiSeq Integration v8.1 Validation and Troubleshooting* on page 2053

The MiSeq Integration Package v8.1 only supports Clarity LIMS v5.1.x to v6.x.

As support for Clarity LIMS 4.x is removed, there is no preconfigured MiSeq Sequencing workflow provided through PreConfigured Workflow Package (PCWP).

Using Illumina Preset Protocols (IPP) v.2.3, install the following workflows:

- MiSeq Sequencing v3.1 workflow
- Library Prep Validation v2.0.4 workflow

The following sections describe the MiSeq Sequencing v3.1 workflow.

For instructions on validating and troubleshooting the Illumina MiSeq Integration Package, see *MiSeq Integration v8.1 Validation and Troubleshooting* on page 2053.

Compatibility

- The MiSeq Sequencing v3.1 workflow is only compatible with MiSeq Integration Package v8.1.
- MiSeq Integration v8.1 is not supported on Clarity LIMS v4.x and v5.0.
- MiSeq Integration v8.1 is only required for MiSeq Control Software (MCS) v4.0. For MCS v3.1 or earlier, do not upgrade to MiSeq Integration v8.1. This upgrade breaks the integration.

Prerequisites and Assumptions

Before samples are assigned to the MiSeq Sequencing v3.1 workflow, make sure that the following prerequisites are completed:

- Samples have been accessioned into Clarity LIMS.
- Samples have been run through QC and library prep.
- Samples have been normalized, and the value is captured in a field called Normalized Molarity (nM).

For more information on sample accessioning, see *Sample Accessioning* and *Upload and Modify Samples* in the Clarity LIMS Core Functionality documentation.

Samples can be assigned to the MiSeq Sequencing v3.1 workflow automatically using a routing script or manually from the Projects & Samples dashboard. See *Assign and Process Samples* in the Clarity LIMS Core Functionality documentation.

Workflows, Protocols, and Steps

The Illumina MiSeq Integration includes the MiSeq Sequencing v3.1 workflow, which contains a single protocol of the same name.

The protocol includes the following steps and automations.

Table 416 MiSeq Sequencing v3.1 Protocol Steps and Automations

Step	Automation
1. Library Pooling (MiSeq v3.1)	• Validate Maximum Number of Samples • Set Next Step - Advance
2. Denature and Dilute (MiSeq v3.1)	• Validate Run Setup and Generate MiSeq SampleSheet • Set Next Step - Advance
3. MiSeq Run (MiSeq v3.1)	• Set Next Step - Advance

Terminology

This integration supports Clarity LIMS v5.1 and later.

As of Clarity LIMS v5, the term user-defined field (UDF) has been replaced with custom field in the user interface. However, the API resource is still called UDF.

There are two types of custom fields:

- Master step fields—Configured on master steps. Master step fields only apply to the following:
  - The master step on which the fields are configured.
  - The steps derived from those master steps.
- Global fields—Configured on entities (eg, submitted sample, derived sample, measurement, etc.). Global fields apply to the entire Clarity LIMS system.

Step 1: Library Pooling (MiSeq v3.1)

In this step, the lab scientist manually places libraries into pools in the Clarity LIMS Placement screen.

Set Next Step - Advance Automation

This automation advances samples to the next step in the protocol. The automation is automatically triggered on exit of the Record Details screen.

The default command line is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-t false \
-h false \
-exp 'nextStep = ::ADVANCE::' \
-log {compoundOutputFileLuid0}"
```

Validate Maximum Number of Samples Automation

This automation checks the maximum number of samples that is allowed in a single pool. The default maximum for Illumina MiSeq Control Software (MCS) is 1536 samples. The automation is automatically triggered on entry of the Pooling screen.

The default command line is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:validateSampleCount -min 1 -max 1536"
```

Master Step Fields

One field is configured on the Library Pooling (MiSeq v3.1) master step. The field displays on the Record Details screen at run time.

Table 417 Library Pooling (MiSeq v3.1) Master Step Field Configuration

Field Name	Field Type	Field Constraints/Options
Comment	Multiline Text	• Required: No • Read Only: No

Global Fields

The following table lists the global fields that are displayed on the Queue and Ice Bucket screens of the Library Pooling (MiSeq v3.1) step. Most fields display in expanded view only.

Table 418 Global Field Configuration for Library Pooling (MiSeq v3.1)

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Configured on submitted sample:			

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Application	Text Dropdown	• Required: No • Read Only: No • Custom Entries: Yes	• TruSeq mRNA sequencing • TruSeq DNA sequencing (large genome *de novo*) • TruSeq DNA sequencing (large genome reseq) • TruSeq DNA sequencing (small genome *de novo*) • TruSeq DNA sequencing (small genome reseq) • Nextera DNA sequencing • TruSeq Custom Amplicon sequencing • ChIP sequencing • Exome sequencing • Mate pair sequencing • Small RNA sequencing
Pooling	Text Dropdown	• Required: No • Read Only: No • Custom Entries: Yes	• Yes • No
Read Length	Text	• Required: No • Read Only: No	Not applicable
Sequencing Coverage	Text	• Required: No • Read Only: No	Not applicable

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Sequencing Method	Text Dropdown	• Required: No • Read Only: No • Custom Entries: Yes	• Single Read • Paired End Read • Indexed Single Read • Indexed Paired End Read
Configured on derived sample:			
Normalized Molarity (nM)	Numeric	• Required: No • Read Only: No	Decimal Places Displayed: 2

User Interaction

1. On the Queue screen, do as follows.
   a. Add the samples to the Ice Bucket.
   b. [Optional] In the Add Control Samples panel, add the PhiX v3 control sample to the Ice Bucket.
   c. Select View Ice Bucket.
2. On the Ice Bucket screen, select Begin Work.
   The Validate Maximum Number of Samples automation script runs. The maximum number of samples allowed is 1536.
3. On the Pool Samples screen, do as follows.
   a. Create a pool of samples by dragging samples into the Pool Creator.
   b. Name the pool or accept the default name (Pool #1).
   c. Select Place Samples.
4. On the Placement screen, do as follows.
   a. Select the pool in the Samples to be Placed area and drag it to the container.
   b. Select Record Details.
5. On the Record Details screen, select Next Steps.
   On the Assign Next Steps screen, next step is already set to Denature and Dilute (MiSeq v3.1).
6. Select Finish Step.

Step 2: Denature and Dilute (MiSeq v3.1)

In this step, pooled libraries are denatured and diluted, and then placed into the reagent cartridge loaded into the MiSeq instrument.

Validate Run Setup and Generate MiSeq SampleSheet Automation

This automation validates the information entered on the Record Details screen, generates the sample sheet (see *Sample Sheet Generation* on page 2045), and attaches the sample sheet to the Denature and Dilute step.

Select a button on the Record Details screen to trigger this automation.

Validation Rules

- The Experiment Name UDF/custom field cannot exceed 40 characters.
- The Experiment Name field can only contain letters, numbers, periods, nonconsecutive spaces, and the following special characters: `~!@#%-_}{.
- If Starling is selected as the variant caller, the Export to gVCF field must be set to Yes.
- If the Read 2 Cycles field is empty or 0, the following rules apply:
  - The Custom Primers field cannot have a selection that contains Read 2.
  - The Adapter Read 2 field cannot have a value.
  - The UMI - Read 2 Length field and the UMI - Read 2 Start From Cycle field cannot have values.
- The UMI - Read 1 Length field and the UMI - Read 1 Start From Cycle field cannot be used independently.
- The UMI - Read 2 Length field and the UMI - Read 2 Start From Cycle field cannot be used independently.
- If the UMI - Read 2 Length field is greater than 0, the UMI - Read 1 Length field must be greater than 0.
- The Adapter Read 1 and Adapter Read 2 fields can only contain ACGT+ characters. The adapter sequence cannot start or end with + or contain more than one +.
- For DNA Enrichment analysis, if the Variant Caller value is Somatic, the Variant Frequency Percentage must be between 1 and 100.
- For DNA Amplicon analysis, if the Variant Caller value is Somatic, the Variant Frequency Percentage field must contain a value between 0.05 and 30.
- If the Aligner value is BWA or TruSeq Amplicon Aligner, an error occurs when the Workflow value is not equal to DNA Amplicon.
- If the Aligner value is BWA-MEM or BWA-Backtrack Legacy, an error occurs when the Workflow value is equal to DNA Amplicon.
- If the Variant Caller value is Starling or GATK, an error occurs when the Workflow value is equal to DNA Amplicon.
- If the Variant Caller value is Somatic, an error occurs when the Workflow value is not equal to DNA Enrichment or DNA Amplicon.

- If the Variant Caller value is Germline, an error occurs when the Workflow value is not equal to DNA Amplicon.

The default command line is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-exp {udf:Validation Script 1} {udf:Validation Script 2}'\
-log {compoundOutputFileLuid3} \
-t true \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p {password} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/MiSeqSamplesheetv3.csv \
-o {compoundOutputFileLuid1}.csv \
-q true \
-destLIMSID {compoundOutputFileLuid1} \
-l {compoundOutputFileLuid2}"
```

There is an 8000 character limit in the Oracle database used for the automation storage. Because of this character limit, the automation splits and saves the validation expressions to the Validation Script 1 and Validation Script 2. These fields are configured as master step fields.

Validation Script 1 Command Line

```
if (step.::Experiment Name::.length() > 40) { fail(::Experiment Name cannot exceed 40 characters.::) }; if (!step.::Experiment Name::.matches(::^(?!.*[ ]{2})[a-zA-Z0-9-_`.~!#@%{ }]+::)) { fail(::Experiment Name contains prohibited characters. Allowed characters are letters, numbers, periods, non-consecutive spaces and the following special characters: `~!@#%-_}{::) }; if (step.::Workflow:: == ::Resequencing:: && step.::Variant Caller:: == ::Starling:: && (!step.hasValue(::Export to gVCF::) || step.::Export to gVCF:: != ::Yes::)) { fail(::Export to gVCF must be set to Yes for Starling variant caller.::) }; if (step.::Workflow:: == ::DNA Enrichment:: && step.::Variant Caller:: == ::Somatic:: && (!step.hasValue(::Variant Frequency Percentage::) || step.::Variant Frequency Percentage:: > 100 || step.::Variant Frequency Percentage:: < 1)) { fail(::In the Variant Frequency Percentage field, please enter values between 1 and 100.::) }; if (step.::Workflow:: == ::DNA Amplicon:: && step.::Variant Caller:: == ::Somatic:: && (!step.hasValue(::Variant Frequency Percentage::) || step.::Variant Frequency Percentage:: > 30 || step.::Variant Frequency Percentage:: < 0.05)) { fail(::In the Variant Frequency Percentage
```

```
field, please enter values between 0.05 and 30.::) }; if (!step.hasValue(::Read
2 Cycles::) || step.::Read 2 Cycles:: == 0) { if (step.::Custom
Primers::.contains(::Read 2::)) { fail(::Custom Primers setting selected is
invalid and can only be used in a Paired-End run.::) }; if (step.hasValue
(::Adapter Read 2::)) { fail(::Adapter Read 2 is only applicable for a Paired-
End run.::) }; if (step.hasValue(::UMI - Read 2 Length::) || step.hasValue(::UMI
- Read 2 Start From Cycle::)) { fail(::UMI - Read 2 Length and UMI - Read 2
Start From Cycle are only applicable for a Paired-End run.::) }; }; if
(step.hasValue(::UMI - Read 1 Length::) && !step.hasValue(::UMI - Read 1 Start
From Cycle::)) { fail(::UMI - Read 1 Start From Cycle must be greater than 0 if
UMI - Read 1 Length is greater than 0.::) }; if (!step.hasValue(::UMI - Read 1
Length::) && step.hasValue(::UMI - Read 1 Start From Cycle::)) { fail(::UMI -
Read 1 Length must be greater than 0 if UMI - Read 1 Start From Cycle is greater
than 0.::) }; if (step.hasValue(::UMI - Read 2 Length::) && !step.hasValue(::UMI
- Read 2 Start From Cycle::)) { fail(::UMI - Read 2 Start From Cycle must be
greater than 0 if UMI - Read 2 Length is greater than 0.::) }; if
(!step.hasValue(::UMI - Read 2 Length::) && step.hasValue(::UMI - Read 2 Start
From Cycle::)) { fail(::UMI - Read 2 Length must be greater than 0 if UMI - Read
2 Start From Cycle is greater than 0.::) }; if (!step.hasValue(::UMI - Read 1
Length::) && step.hasValue(::UMI - Read 2 Length::)) { fail(::UMI - Read 1
Length must be greater than 0 if UMI - Read 2 Length is greater than 0.::) }; if
(step.hasValue(::Adapter Read 1::) && !step.::Adapter Read 1::.matches(::^
[ACGT]+(\\+[ACGT]+){0,1}$::)) { fail(::Adapter Read 1 contains prohibited
characters. Allowed characters are: ACGT+ and the adapter sequence cannot start
or end with + or contain more than one +.::) }; if (step.hasValue(::Adapter Read
2::) && !step.::Adapter Read 2::.matches(::^[ACGT]+(\\+[ACGT]+){0,1}$::)) { fail
(::Adapter Read 2 contains prohibited characters. Allowed characters are: ACGT+
and the adapter sequence cannot start or end with + or contain more than one
+.::) };
```

Validation Script 2 Command Line

```
if ((step.::Aligner:: == ::BWA:: || step.::Aligner:: == ::TruSeq Amplicon
Aligner::) && step.::Workflow:: != ::DNA Amplicon::) { fail(::Aligner field
contains invalid value. Please refer to the \u005c\u0022Applicable analysis
fields for the selected Workflow:\u005c\u0022 section for more information.::)
}; if ((step.::Aligner:: == ::BWA-MEM:: || step.::Aligner:: == ::BWA-Backtrack
Legacy::) && step.::Workflow:: == ::DNA Amplicon::) { fail(::Aligner field
contains invalid value. Please refer to the \u005c\u0022Applicable analysis
fields for the selected Workflow:\u005c\u0022 section for more information.::)
}; if ((step.::Variant Caller:: == ::Starling:: || step.::Variant Caller:: ==
::GATK::) && step.::Workflow:: == ::DNA Amplicon::) { fail(::Variant Caller
```


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```
field contains invalid value. Please refer to the \u005c\u0022Applicable
analysis fields for the selected Workflow:\u005c\u0022 section for more
information.::) }; if (step.::Variant Caller:: == ::Somatic:: &&
step.::Workflow:: != ::DNA Enrichment:: && step.::Workflow:: != ::DNA
Amplicon::) { fail(::Variant Caller field contains invalid value. Please refer
to the \u005c\u0022Applicable analysis fields for the selected
Workflow:\u005c\u0022 section for more information.::) }; if (step.::Variant
Caller:: == ::Germline:: && step.::Workflow:: != ::DNA Amplicon::) { fail
(::Variant Caller field contains invalid value. Please refer to the
\u005c\u0022Applicable analysis fields for the selected Workflow:\u005c\u0022
section for more information.::) }; step.::Indel-Realignment-Key:: = ::::;
step.::Indel-Realignment-Value:: = ::::; step.::Aligner-Key:: = ::::;
step.::Aligner-Value:: = ::::; step.::Variant-Caller-Value:: = ::::; if
(step.::Workflow:: == ::DNA Amplicon::) { if (step.::Indel Realignment:: ==
::Yes::) { step.::Indel-Realignment-Key:: = ::variantcallerrealignindels::;
step.::Indel-Realignment-Value:: = ::1::; }; else if (step.::Indel Realignment::
== ::No::) { step.::Indel-Realignment-Key:: = ::variantcallerrealignindels::;
step.::Indel-Realignment-Value:: = ::0::; }; if (step.::Aligner:: == ::TruSeq
Amplicon Aligner::) { step.::Aligner-Key:: = ::aligner::; step.::Aligner-Value::
= ::Amplicon::; }; else if (step.::Aligner:: == ::BWA::) { step.::Aligner-Key::
= ::aligner::; step.::Aligner-Value:: = ::BWA::; }; }; if (step.::Workflow:: !=
::DNA Amplicon::) { if (step.::Indel Realignment:: == ::Yes::) { step.::Indel-
Realignment-Key:: = ::indelrealignment::; step.::Indel-Realignment-Value:: =
::GATK::; }; else if (step.::Indel Realignment:: == ::No::) { step.::Indel-
Realignment-Key:: = ::indelrealignment::; step.::Indel-Realignment-Value:: =
::None::; }; if (step.::Aligner:: == ::BWA-Backtrack Legacy::) { step.::Aligner-
Key:: = ::runbwaaln::; step.::Aligner-Value:: = ::1::; }; else if
(step.::Aligner:: == ::BWA-MEM::) { step.::Aligner-Key:: = ::runbwaaln::;
step.::Aligner-Value:: = ::0::; }; }; if (step.hasValue(::Variant Caller::)) {
if (step.::Variant Caller:: == ::None::) { step.::Variant-Caller-Value:: = ::::;
}; if (step.::Variant Caller:: == ::GATK::) { step.::Variant-Caller-Value:: =
::GATK::; }; else if (step.::Variant Caller:: == ::Starling::) { step.::Variant-
Caller-Value:: = ::Starling::; }; else if (step.::Variant Caller:: ==
::Germline::) { step.::Variant-Caller-Value:: = ::PiscesGermline::; }; else if
(step.::Variant Caller:: == ::Somatic:: && step.::Workflow:: == ::DNA
Amplicon::) { step.::Variant-Caller-Value:: = ::PiscesSomatic::; }; else if
(step.::Variant Caller:: == ::Somatic:: && step.::Workflow:: != ::DNA
Amplicon::) { step.::Variant-Caller-Value:: = ::Somatic::; }; }; if
(step.hasValue(::Manifest::)) { step.::Manifest-Section:: = ::[Manifests]::; };
else { step.::Manifest-Section:: = ::::; }
```

Set Next Step - Advance Automation

This automation advances samples to the next step in the protocol. The automation is automatically triggered by exiting the Record Details screen.

The default command line is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-t false \
-h false \
-exp 'nextStep = ::ADVANCE::' \
-log {compoundOutputFileLuid3}"
```

Master Step Fields

Most fields configured on the Denature and Dilute (MiSeq v3.1) step display on the Record Details screen in the Step Data table.

These fields are manually populated at run time (see *User Interaction* on page 2041). The values are then used to generate the sample sheet.

Table 419 Denature & Dilute (MiSeq v3.1) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Drop-Down Items
Adapter Read 1	Text	• Required: No • Read Only: No	Not applicable
Adapter Read 2	Text	• Required: No • Read Only: No	Not applicable
Aligner	Text drop-down	• Required: No • Read Only: No • Custom Entries: No	• None (default) • BWA-MEM • BWA-Backtrack Legacy • BWA • TruSeq Amplicon Aligner

Field Name	Field Type	Options	Additional Options and Drop-Down Items
Aligner-Key	Text	• Required: No • Read Only: Yes • Custom Entries: No	Hidden, used for sample sheet generation Do not delete / modify this field. These actions will break sample sheet generation automation.
Aligner-Value	Text	• Required: No • Read Only: Yes • Custom Entries: No	
Annotation	Text drop-down	• Required: No • Read Only: No • Custom Entries: No	• None • RefSeq • Ensembl
Applicable analysis fields for the selected Workflow	Multiline Text	• Required: No • Read Only: Yes	Select a value from the drop-down list in the upper-right corner.
Comment	Multiline Text	• Required: No • Read Only: No	Not applicable
Custom Primers	Text drop-down	• Required: No • Read Only: No • Custom Entries: No	• None (Default) • Read 1 • Index • Read 2 • Read 1, Index • Read 1, Index, Read 2 • Read 1, Read 2 • Index, Read 2
Description	Text	• Required: No • Read Only: No	Not applicable
Experiment Name	Text	• Required: Yes • Read Only: No	Not applicable

Field Name	Field Type	Options	Additional Options and Drop-Down Items
Export to gVCF	Text drop-down	• Required: No • Read Only: No • Custom Entries: No	• None (default) • Yes • No
Flag PCR Duplicates	Text	• Required: No • Read Only: No • Custom Entries: No	• None (default) • Yes • No
Genome Folder	Text	• Required: No • Read Only: No	Not applicable
Indel Realignment	Text drop-down	• Required: No • Read Only: No • Custom Entries: No	• None (default) • Yes • No
Indel Repeat Filter Cutoff	Text drop-down	• Required: No • Read Only: No • Custom Entries: No	• None (default) • Yes • No
Indel-Realignment-Key	Text	• Required: No • Read Only: Yes • Custom Entries: No	Hidden, used for sample sheet generation Do not delete / modify this field. These actions will break sample sheet generation automation.
Indel-Realignment-Value	Text	• Required: No • Read Only: Yes • Custom Entries: No	
Manifest	Text	• Required: No • Read Only: No	Not applicable
Manifest Padding	Text Dropdown	• Required: No • Read Only: No • Custom Entries: No	• None (default) • 0 • 50 • 100 • 150 • 200 • 250

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Field Name	Field Type	Options	Additional Options and Drop-Down Items
Manifest-Section	Text	• Required: No • Read Only: Yes • Custom Entries: No	Hidden, used for sample sheet generation Do not delete / modify this field. These actions will break sample sheet generation automation.
Picard HS Metric Reporting	Text Dropdown	• Required: No • Read Only: No • Custom Entries: No	• None (default) • Yes • No
Read 1 Cycles	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 251 (Default) • 151 • 101 • 76 • 51 • Range From: 1 to 1000 • Decimal Places Displayed: 0
Read 2 Cycles	Numeric Dropdown	• Required: No • Read Only: No • Custom Entries: Yes	• 251 • 151 • 101 • 76 • 51 • Range From: 0 to 1000 • Decimal Places Displayed: 0
Read Stitching	Text Dropdown	• Required: No • Read Only: No • Custom Entries: No	• None (default) • Yes • No
Reverse Complement	Text Dropdown	• Required: No • Read Only: No • Custom Entries: No	• None (default) • Yes • No

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Field Name	Field Type	Options	Additional Options and Drop-Down Items
UMI - Read 1 Length	Numeric	• Required: No • Read Only: No	• Minimum value: 1
UMI - Read 1 Start From Cycle	Numeric	• Required: No • Read Only: No	• Minimum value: 1
UMI - Read 2 Length	Numeric	• Required: No • Read Only: No	• Minimum value: 1
UMI - Read 2 Start From Cycle	Numeric	• Required: No • Read Only: No	• Minimum value: 1
Validation Script 1	Multiline text	• Required: Yes • Read Only: No	Hidden, used for automation Do not delete / modify this field. These actions will break sample sheet generation automation.
Validation Script 2	Multiline text	• Required: Yes • Read Only: No	
Variant Caller	Text Dropdown	• Required: No • Read Only: No • Custom Entries: No	• None (Default) • GATK • Germline • Somatic • Starling
Variant Caller Depth Filter	Numeric	• Required: No • Read Only: No	• Minimum value: 10 • Maximum value: 10000
Variant Quality Filter	Numeric	• Required: No • Read Only: No	• Minimum value: 2 • Maximum value: 1000
Variant Frequency Percentage	Numeric	• Required: No • Read Only: No	• Minimum value: 0.05 • Maximum value: 100

Field Name	Field Type	Options	Additional Options and Drop-Down Items
Variant-Caller-Value	Text	• Required: No • Read Only: Yes • Custom Entries: No	Hidden, used for sample sheet generation Do not delete / modify this field. These actions will break sample sheet generation automation.
Workflow	Text Dropdown	• Required: Yes • Read Only: No • Custom Entries: No	• GenerateFASTQ • LibraryQC • Resequencing • DNA Enrichment • DNA Amplicon

Groups of Defaults

The following groups of defaults are configured for Denature and Dilute (MiSeq v3.1) step fields:

Resequencing

Field	Configuration
Workflow	Resequencing
Read 1 Cycles	251
Custom Primers	None
Aligner	BWA-MEM
Annotation	None
Export to gVCF	No
Flag PCR Duplicates	Yes
Genome Folder	Required
Indel Realignment	No
Indel Repeat Filter Cutoff	None
Manifest	None

Field	Configuration
Manifest Padding	None
Picard HS Metric Reporting	None
Reverse Complement	None
Read Stitching	None
Variant Caller	GATK
Applicable analysis fields for the selected workflow	• Aligner (BWA-Backtrack Legacy, BWA-MEM) • Export to gVCF • Flag PCR Duplicates • Genome Folder (fill in the folder path) • Indel Realignment • Variant Caller (Starling, GATK)

Library QC

Field	Configuration
Workflow	Library QC
Read 1 Cycles	251
Custom Primers	None
Aligner	None
Annotation	None
Export to gVCF	None
Flag PCR Duplicates	Yes
Genome Folder	Required
Indel Realignment:	None
Indel Repeat Filter Cutoff	None
Manifest	None
Manifest Padding	None
Picard HS Metric Reporting	None
Reverse Complement	None
Read Stitching	None

Field	Configuration
Variant Caller	None
Applicable analysis fields for the selected Workflow	• Flag PCR Duplicates • Genome Folder (fill in the folder path)

GenerateFastQ

Field	Configuration
Workflow	GenerateFastQ
Read 1 Cycles	251
Custom Primers	None
Aligner	None
Annotation	None
Export to gVCF	None
Flag PCR Duplicates	None
Genome Folder	None
Indel Realignment	None
Indel Repeat Filter Cutoff	None
Manifest	None
Manifest Padding	None
Picard HS Metric Reporting	None
Reverse Complement	None
Read Stitching	None
Variant Caller	None
Applicable analysis fields for the selected Workflow	None

DNA Enrichment

Field	Configuration
Workflow	DNA Enrichment
Read 1 Cycles	251

Field	Configuration
Custom Primers	None
Aligner	BWA-MEM
Annotation	None
Export to gVCF	None
Flag PCR Duplicates	Yes
Genome Folder	Required
Indel Realignment	Yes
Indel Repeat Filter Cutoff	None
Manifest	Required
Manifest Padding	150
Picard HS Metric Reporting	No
Reverse Complement	None
Read Stitching	None
Variant Caller	Starling
Applicable analysis fields for the selected Workflow	• Aligner (BWA-Backtrack Legacy, BWA-MEM) • Flag PCR Duplicates • Genome Folder (fill in the folder path) • Indel Realignment • Indel Repeat Filter Cutoff (if Variant Caller is Somatic) • Manifest (fill in the file name) • Manifest Padding • Picard HS Metric Reporting • Variant Caller (Starling, Somatic, GATK) • Variant Frequency Percentage (if Variant Caller is Somatic)

DNA Amplicon

Field	Configuration
Workflow	DNA Amplicon
Read 1 Cycles	251
Custom Primers	None
Aligner	BWA
Annotation	RefSeq
Export to gVCF	None
Flag PCR Duplicates	None
Genome Folder	Required
Indel Realignment	Yes
Indel Repeat Filter Cutoff	None
Manifest	Required
Manifest Padding	None
Picard HS Metric Reporting	None
Reverse Complement	None
Read Stitching	None
Variant Caller	Germline
Variant Caller Depth Filter	10
Variant Quality Filter	30

Field	Configuration
Applicable analysis fields for the selected Workflow	• Aligner (BWA, TruSeq Amplicon Aligner) • Annotation (RefSeq, Ensembl) • Genome Folder (fill in the folder path) • Indel Realignment • Manifest (fill in the file name) • Read stitching (if Aligner is TruSeq Amplicon Aligner) • Variant Caller (Germline, Somatic) • Variant Caller Depth Filter (10–10000) • Variant Frequency Percentage (if Variant Caller is Somatic) • Variant Quality Filter (2–1000)

Global Fields

The following table shows the global fields that are configured to display on the Queue, Ice Bucket, and Record Details screens of the Denature and Dilute (MiSeq v3.1) step:

The Submitted Sample field, Progress, (added from previous MiSeq workflow) is obsolete in MiSeq v3.1.

Table 420 Global Field Configuration for Denature and Dilute (MiSeq v3.1) Step

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Configured on submitted sample:			

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Read Length *Displays on Queue & Ice Bucket screens*	Text	• Required: No • Read Only: No	Not applicable
Sequencing Method *Displays on Queue & Ice Bucket screens*	Text	• Required: No • Read Only: No • Custom Entries: Yes	• Single Read • Paired End Read • Indexed Single Read • Indexed Paired End Read
Configured on derived sample:			
Final Loading Concentration *Displays on Record Details screen*	Numeric Dropdown	• Required: Yes • Read Only: No • Custom Entries: Yes	• 225 • 400

Step File Placeholders

Placeholders for the following files are configured on the Record Details screen of the Denature and Dilute (MiSeq v3.1) step:

- Lab Tracking Form (manually generated)
- MiSeq SampleSheet (autogenerated)
- MiSeq SampleSheet Generation Log (autogenerated)
- Log File (autogenerated)

Table 421 Sample Sheet Files and Placeholders Generated by Denature and Dilute (MiSeq v3.1) Step

Item	Description
Lab Tracking Form placeholder	This placeholder in Clarity LIMS allows the lab scientist to attach a lab-specific tracking form to the step manually.

Item	Description
MiSeq SampleSheet	This CSV file is automatically generated by Clarity LIMS for use with the MiSeq system. The file can be opened as a text file or an Excel spreadsheet.
MiSeq SampleSheet Generation Log	This log file is automatically generated by Clarity LIMS. The log file captures any errors that Clarity LIMS might encounter when generating the sample sheet.
Log File	This log file is automatically generated by Clarity LIMS. The log file captures the status of the EvaluateDynamicExpression script that is invoked by the Set Next Step - Advance automation.

User Interaction

1. On the Queue screen, add the pool to the Ice Bucket, and then select View Ice Bucket.
2. On the Ice Bucket screen, select Begin Work.
3. On the Placement screen, do as follows.
   a. Scan the barcode of the MiSeq reagent cartridge into the MiSeq Reagent Cartridge field.
   b. Place the pool of samples into the reagent cartridge.
   c. Select Record Details.
4. On the Record Details screen, do as follows.
   a. Under Reagent Lot Tracking, select the reagent lot used in the step.
   If the reagent lot is not listed, add/activate the lot on the Reagents and Controls screen.
   b. Populate the fields in the Step Details table, as needed. Workflow, Experiment Name, and Read 1 Cycles are required fields. For details on how to populate the fields by using the preset drop-down list, see *MiSeq Integration v8.1 Validation and Troubleshooting* on page 2053.
   c. In the Sample Details table, enter the Final Loading Concentration. Select from the following preset options or enter a different value.

- 225 (for PCR-free workflows)
- 400 (for Nano workflows)

d. Select Validate Run Setup and Generate MiSeq SampleSheet.
Clarity LIMS generates the sample sheet and attaches it and a log file to placeholders in the Files area of the Record Details screen.

e. Download the files and validate their format and content.

5. Select Next Steps.
On the Assign Next Steps screen, samples are already assigned to the MiSeq Run (MiSeq v3.1) step.
6. Select Finish Step.

Step 3: MiSeq Run (MiSeq v3.1)

Set Next Step - Advance Automation

This automation advances samples to the next step in the protocol. The automation is automatically triggered by exiting the Record Details screen.

The default command line is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-t false \
-h false \
-exp 'nextStep = ::ADVANCE::' \
-log {compoundOutputFileLuid5}"
```

Master Step Fields

There are 18 fields configured on the MiSeq Run (MiSeq v3.1) step. These fields display on the Record Details screen at run time.

The read-only fields are automatically populated at the end of the run.

Table 422 MiSeq Run (MiSeq v3.1) Master Step Field Configuration

Field Name	Field Type	Options	Additional Options and Drop-Down Items
Chemistry	Text	• Required: No • Read Only: Yes	Not applicable

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Field Name	Field Type	Options	Additional Options and Drop-Down Items
Comment	Multiline Text	• Required: No • Read Only: No	Not applicable
Experiment Name	Text	• Required: No • Read Only: Yes	Not applicable
Finish Date	Date	• Required: No • Read Only: Yes	Not applicable
Flow Cell ID	Text	• Required: No • Read Only: Yes	Not applicable
Flow Cell Version	Text	• Required: No • Read Only: Yes	Not applicable
Index 1 Read Cycles	Numeric	• Required: No • Read Only: Yes	Decimal Places Displayed: 0
Index 2 Read Cycles	Numeric	• Required: No • Read Only: Yes	Decimal Places Displayed: 0
Output Folder	Text	• Required: No • Read Only: Yes	Not applicable
PR2 Bottle ID	Text	• Required: No • Read Only: Yes	Not applicable
Read 1 Cycles	Numeric	• Required: No • Read Only: Yes	Decimal Places Displayed: 0
Read 2 Cycles	Numeric	• Required: No • Read Only: Yes	Decimal Places Displayed: 0
Reagent Cartridge ID	Text	• Required: No • Read Only: Yes	Not applicable
Reagent Cartridge Part #	Text	• Required: No • Read Only: Yes	Not applicable
Run ID	Text	• Required: No • Read Only: Yes	Not applicable
Run Type	Text	• Required: No • Read Only: Yes	Not applicable
Status	Text	• Required: No • Read Only: Yes	Not applicable

Field Name	Field Type	Options	Additional Options and Drop-Down Items
Workflow	Text	• Required: No • Read Only: Yes	Not applicable

Global Fields

There are several sample and measurement global fields that are displayed on the Record Details screen of the MiSeq Run (MiSeq v3.1) step. These fields are autopopulated at the end of the sequencing run.

For more information, see *User Interaction* on page 2044 and *Sequencing Results Parsing* on page 2045.

Step File Placeholders

Placeholders for the following files are configured on the Record Details screen of the MiSeq Run (MiSeq v3.1) step:

- Illumina Run Report (autogenerated)
- Link to Run Folder (autogenerated)
- Run Parameters (autogenerated)
- Run Info (autogenerated)
- Lab Tracking Form (manually generated)
- Log File (autogenerated)

For details, see *Generated and Captured Files* on page 2045.

User Interaction

1. On the Queue screen, add the pool to the Ice Bucket, and then select View Ice Bucket.
2. On the Ice Bucket screen, select Begin Work.
3. On the Record Details screen, the fields in the table are read-only. After the run completes, the integration automatically performs the following actions:
   - Populates the fields.
   - Attaches files to the Illumina Run Report, Link to Run Folder, Run Parameters, and Run Info placeholders.
   - Populates the fields in the Sample Details table.
4. Select Next Steps.
   On the Assign Next Steps screen, the next step is already set to Mark protocol as complete.

5. Select Finish Step.

Sample Sheet Generation

Sample sheet generation occurs in the Denature & Dilute (MiSeq v3.1) step. This step places samples on the container loaded in the system.

The default configuration provides only the Validate Run Setup and Generate MiSeq SampleSheet automation. This automation uses the Template File Generator (`DriverFileGenerator.jar`) and a template file to generate a CSV format file for use with the MiSeq Control Software (MCS).

The sample sheet content is determined by the fields on the Record Details screen of the step in the Step Data table. The values entered into these fields are used to populate the sample sheet.

To customize the template used to create the sample sheet, you can insert additional columns.

The following additional details are available:

- For a sample template that you can donwload and customize for the lab, see *Illumina Instrument Sample Sheets (NGS v5.17 & later)* on page 2542.
- For instructions on customizing the template, see *Creating Template Files* on page 2454.

Sequencing Results Parsing

The MiSeq Run (MiSeq v3.1) step records information for the flow cell lane and generates a report summarizing the results. In addition, run parameters, run info, and a link to the run folder are automatically captured.

Generated and Captured Files

The following table describes the run information files, reports, placeholders, and links that Clarity LIMS automatically generates or captures during a sequencing run:

Table 423 Run Information Generated or Captured by MiSeq Run (MiSeq v3.1) Step

Item	Description
Run Info Run Parameters	These XML files are automatically captured by Clarity LIMS from the run folder of the system. They include the key run parameters that are parsed out into step UDFs/custom fields in Clarity LIMS.
Link to Run Folder	This link is the path to the network run folder where the data that was captured from the system during the run is stored. The link is automatically generated by Clarity LIMS.

Item	Description
Illumina Run Report	This report provides key information about the run and the samples on the flow cell. The report is automatically generated by Clarity LIMS. Information includes the flow cell ID, run directory location, and primary analysis metrics for the sequencing run. Information is summarized per flow cell lane for the entire run and individual reads for paired-end runs. These metrics are compared against the per lane averages of the sequencing run. The per lane averages are calculated using metrics from the last five sequencing runs. Any values outside of one standard deviation are highlighted.
Lab Tracking Form placeholder	This placeholder in Clarity LIMS allows you to manually attach a lab-specific tracking form to the step.

Metadata

The following list shows metadata that Clarity LIMS automatically captures from the Illumina sequencing software as part of a sequencing run. This information is gathered from various run result files and events.

- Chemistry
- Experiment Name (entered in software)
- Finish Date (run completion date)
  - If the End Run event contains a date in the format YYYY-MM-DD, Finish Date is set to the date in the event file.
  - If the End Run event does not contain a date or the date is in the wrong format, Finish Date is set to the date when the event file is processed.
- Flow Cell ID
- Flow Cell Version
- Index 1 Read Cycles (intended Index cycles)
- Index 2 Read Cycles (intended Index cycles)
- Output Folder (run folder root)
- PR2 Bottle ID
- Reagent Cartridge ID
- Reagent Cartridge Part #
- Read 1 Cycles

- Read 2 Cycles
- Run ID (the unique run ID)
- Run Type
- Status (current status of the sequencing run on the instrument)
- Workflow

Primary Analysis Metrics

The following table lists the real-time analysis (RTA) primary analysis metrics Clarity LIMS automatically captures and records per read, for samples in each flow cell lane. These metrics are captured upon run completion and are stored as fields in the Sample Details table on the Record Details screen.

To see both per read and per lane metrics, expand the output.

Table 424 RTA Primary Analysis Metrics Captured by MiSeq Run (MiSeq v3.1) Step

Per Read LIMS Field Name *(Stored on derived sample/analyte input to the step)*	Per Lane LIMS Field Name *(Stored in measurement placeholders in the Sample Details table on the Record Details screen)*
% Aligned R1	% Aligned R1
% Aligned R2	% Aligned R2
% Bases >=Q30 R1	% Bases >=Q30 R1
% Bases >=Q30 R2	% Bases >=Q30 R2
% Error Rate R1	% Error Rate R1
% Error Rate R2	% Error Rate R2
Not applicable	% Phasing R1
Not applicable	% Phasing R2
Not applicable	% Prephasing R1
Not applicable	% Prephasing R2
Not applicable	%PF R1
Not applicable	%PF R2
Not applicable	Cluster Density (K/mm^2) R1
Not applicable	Cluster Density (K/mm^2) R2
Intensity Cycle 1 R1	Intensity Cycle 1 R1
Intensity Cycle 1 R2	Intensity Cycle 1 R2

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Per Read LIMS Field Name *(Stored on derived sample/analyte input to the step)*	Per Lane LIMS Field Name *(Stored in measurement placeholders in the Sample Details table on the Record Details screen)*
Not applicable	Reads PF (M) R1
Not applicable	Reads PF (M) R2
Yield PF (Gb) R1	Yield PF (Gb) R1
Yield PF (Gb) R2	Yield PF (Gb) R2

How It Works

1. The sequencing service runs on the Clarity LIMS server. The service detects event files that instrument RTA produces as the run progresses. The event files let the service know where to find the run data.
2. As the run data are written out and the End Run event is detected, the following events occur:
   - The data are matched to the step based on the reagent cartridge ID that was entered or scanned on the Denature and Dilute (MiSeq v3.1) step.
   - Read-only field values on the Record Details screen are populated accordingly.
3. When the service has finished processing the end run event and updating the fields in Clarity LIMS, the sequencing service generates the report and attaches it to the step.

User Interaction

1. Manually start the step. Leave the step in progress until the sequencing service has finished processing the run and the run report is generated and attached.
2. After the run has completed, verify the following:
   - The read-only fields on the Record Details screen are populated.
   - The Illumina Run Report is generated and attached.
   - The Link to Run Folder is generated and attached.
   - The Run Parameters and Run Info files are attached.

   Leave the step as in progress until the Illumina Run Report has been attached to the step in Clarity LIMS.
3. [Optional] In the Files section, upload the Lab Tracking Form.
4. Select Next Steps.
   On the Assign Next Steps screen, the next step is already set to Mark protocol as complete. The Log File is attached when the next step for samples has been assigned by the Next Step - Advance automation.

5. Select Finish Step.

Scripts and Files Installed

This integration requires components installed with the Illumina Preset Protocols (IPP).

The Illumina MiSeq Integration Package v8.1 RPM installs the scripts and files listed in the following table.

Component	Location & Description
`configure_extensions_miseq_sequencingservice-v8.sh`	/opt/gls/clarity/config/ Script that installs the service properties in the database.
`log4j.xml`	/opt/gls/clarity/extensions/Illumina_MiSeq/v8/SequencingService/conf File containing the settings for logging the sequencing jar.
`miseq-sequencing-v8.jar`	/opt/gls/clarity/extensions/Illumina_MiSeq/v8/SequencingService Jar file containing API-based Clarity LIMS extensions used for capturing run results and report generation.
InterOp libraries	/opt/gls/clarity/extensions/Illumina_MiSeq/v8/lib Illumina shared library for parsing InterOp data files.
`MiSeqSamplesheetv3.csv` (Installed by the PCWP or Illumina Preset Protocols (IPP))	/opt/gls/clarity/extensions/conf/driverfiletemplates Template file used for file generation.

Database Properties Installed

The database properties installed in the Illumina MiSeq Integration v8.1 are described in the following section.

The integration also installs several additional database properties, each with the 99 suffix appended to their name, that are not listed in the table. These properties are intended for use by the Clarity LIMS support team in automated validation tests.

Sequencing runs are matched using the reagent cartridge ID and the base name of the sequencing step, MiSeq Run (MiSeq v3.1).

Do not change the base name. The sequencing service that captures sequencing run results expects this name. The base name is stored in the `sequenceProcessBaseName` database property. If the name is changed without the database property being updated, the reagent cartridge ID <-> sequencing step base name matching system fails.

If you need to rename the step, for example to add a version number, modify the text after the base name portion. This part of the name is not used in the matching system. For example, change MiSeq Run (MiSeq v3.1) to MiSeq Run (MiSeq v3.1)-Updated.

Table 425 Database Properties Installed with the Illumina MiSeq Integration Package

Property	Description	Default Value
`miseq.v8.seqservice.sequenceProcessBaseName`	Sequencing process type/master step base display name. Partial matching is used to look up the process type/master step. This property is added/updated by Illumina Preset Protocols (IPP) during the workflow installation.	MiSeq Run (MiSeq v3.1)
`miseq.v8.seqservice.eventFileDirectory.1`	A network location monitored for event files, eg, `/mnt/illumina/gls_events/`	/mnt/gls_events
`miseq.v8.seqservice.netPathPrefixSearch.1`	The network directory prefix contained in the event file.	\\nas\network\run_data
`miseq.v8.seqservice.netPathPrefixReplace.1`	The mapped network directory mount name on the server used to access the run data directory, eg, `/mnt/network/data`	/mnt/run_data

Property	Description	Default Value
`miseq.v8.seqservice.eventFileDirectorySuffixes`	A list of eventFileDirectory path entries to monitor for event files. The value is one or more comma-separated integers.	99 Configured on install to point to 1
`miseq.v8.seqservice.netPathPrefixSearchReplaceSuffixes`	A list of netPathPrefix search and replace entries for transforming Windows to Linux network paths. The value is one or more comma-separated integers.	99 Configured on install to point to 1
`miseq.v8.seqservice.runReportViewsVersion`	The current version of the Run Report views in the database. The value 0 represents the state before the views are created. This property is automatically updated by the run report.	0

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Property	Description	Default Value
`miseq.v8.seqservice.ignoreUnmatchedContainerIds`	A flag indicating if event files that cannot be matched to reagent cartridges in Clarity LIMS should be archived after a certain period (true) or continually reprocessed (false).	False To prevent the gls_events file directory from becoming cluttered, it is recommended that the value of this property is set to true
`miseq.v8.seqservice.ignoreUnmatchedContainerIdsWaitDays`	The number of days between when the event is created and the event file is archived.	14
`miseq.v8.seqservice.synchronizationPeriod`	Invocation period in seconds.	60

You can configure support for multiple, identical seqservice.netPathPrefixSearch property values. For more information, see *Configure Multiple Identical netPathPrefixSearch Values* on page 2596

Consumables Installed

Reagent Categories and Kits

Reagent categories or label groups are installed with the Preconfigured Workflow Package (PCWP) workflow configuration slices.

The MiSeq Reagent Kit is included in the Illumina MiSeq Integration.

Control Types

The PhiX v3 control type is included in the Illumina MiSeq Integration.

Container Types

The MiSeq Reagent Cartridge container type is included in the Illumina MiSeq Integration.

All one-dimensional container types with both numeric rows and numeric columns are supported.

Instrument Integration

To make sure that the Illumina instrument warranty remains valid, the instrument integration must be performed and maintained by the Clarity LIMS Support team. To perform this integration, the Support team requires remote access to the system while it is idle.

The following steps are performed by the Clarity LIMS Support team when configuring the sequencing for use with the Illumina MiSeq Integration.

1. Create a directory on the local computer to hold the batch files. These batch files write event files to the network attached storage (NAS) shares.
2. Create a directory on the NAS to hold the event files.
3. Modify Illumina software configuration files to call the batch files that create the event files.
4. Update sequencing service default database properties to match the specifics of the installation.

Rules and Constraints

The Illumina MiSeq Integration operates with the following constraints:

- The reagent cartridge ID must be unique. There should not be multiple reagent cartridge containers in the system with the same name.
- The reagent cartridge ID must be scanned as the reagent cartridge Container Name on the Denature & Dilute (MiSeq v3.1) step.

MiSeq Integration v8.1 Validation and Troubleshooting

The following sections explain how to validate the installation of the Illumina MiSeq Integration Package v8.1.

The validation involves the following processes:

- Running samples through the Library Prep Validation v2.0.4 workflow. The workflow contains a single-step protocol that models the library prep required to produce normalized libraries. At the end of the step, the normalized libraries automatically advance to the MiSeq Sequencing v3.1 workflow.
- Running normalized libraries through the MiSeq Sequencing v3.1 workflow. This process validates the following information:
  - Automated generation of a sample sheet for use with the MiSeq Control Software (MCS) and Clarity LIMS Product Analytics.
  - Automated tracking of the MiSeq sequencing run and parsing of run statistics into Clarity LIMS, including the following information:
    - Run status and metrics of sequencing run
    - Sequencing run parameters

- Real-Time Analysis (RTA) v1 run directory location and other run-specific information

Activate Workflow, Create Project, Add and Assign Samples

The following steps prepare Clarity LIMS to run samples through the Library Prep Validation and MiSeq Sequencing v3.1 workflows.

1. In the Clarity LIMS Configuration area, select each workflow and change the Workflow Status toggle to Active.
2. On the Projects & Samples screen, create a project. Add samples to the project.
3. Assign the samples to the Library Prep Validation workflow.

Library Prep Validation Protocol

This single-step protocol models the library prep required to produce normalized libraries that are ready for the MiSeq Sequencing v3.1 workflow.

Run Library Prep Validation Step

1. In Lab View, locate the Library Prep Validation protocol. Samples are queued for the Library Prep Validation step. Select the step to proceed to the Queue screen.
2. On the Queue screen, add the samples to the Ice Bucket, and then select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Placement screen, do as follows.
   a. In the container field in the Placed Samples area, scan or enter the barcode of the destination container.
   b. Select samples from the Samples to be Placed area and drag them to the container wells on the right. Repeat until all samples are placed.
   c. Select Add Labels.
5. On the Add Labels screen, do as follows.
   a. To assign labels, select a label and drag it to a container well. Repeat as needed.
   b. Select Record Details.
6. On the Record Details screen, do as follows.
   a. Select all the samples in the Sample Details table.
   b. In the Sequencing Instrument drop-down list, select MiSeq.
   c. Select Apply to set this value for all selected samples.
   d. Select Next Steps.

   On the Assign Next Steps screen, the next step is automatically set to Remove from Workflow. This setting is required by the Routing script automation.
7. Select Finish Step.

The Routing script automation is triggered. Samples are routed to the first step in the MiSeq Sequencing v3.1 protocol—Library Pooling (MiSeq v3.1).

MiSeq Sequencing v3.1 Protocol

Run Library Pooling (MiSeq v3.1)

1. In Lab View, locate the MiSeq Sequencing v3.1 protocol. Samples are queued for the Library Pooling (MiSeq v3.1) step. Select the step to proceed to the Queue screen.
2. On the Queue screen, do as follows.
   a. Add the samples to the Ice Bucket.
   b. In the Add Control Samples panel, add the PhiX v3 control sample to the Ice Bucket.
   c. Select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
   The Validate Maximum Number of Samples automation script runs. The maximum number of samples allowed is 1536.
4. On the Pool Samples screen, do as follows.
   a. Create a pool of samples by dragging samples into the Pool Creator.
   b. Name the pool or accept the default name (Pool #1).
   c. Select Place Samples.
5. On the Placement screen, do as follows.
   a. Select the pool from the Samples to be Placed area and drag it to the container.
   b. Select Record Details.
6. On the Record Details screen, select Next Steps.
   On the Assign Next Steps screen, next step is automatically set to Denature and Dilute (MiSeq v3.1).
7. Select Finish Step.

Run Denature and Dilute (MiSeq v3.1)

1. In Lab View, locate the MiSeq Sequencing v3.1 protocol. The pooled samples are queued for the Denature and Dilute (MiSeq v3.1) step. Select the step to proceed to the Queue screen.
2. On the Queue screen, add the pool to the Ice Bucket, and then select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Placement screen, do as follows.
   a. Scan the MiSeq reagent cartridge barcode into the MiSeq Reagent Cartridge field.
   b. Place the pool of samples into the reagent cartridge.
   c. Select Record Details.
5. On the Record Details screen, do as follows.

a. In Reagent Lot Tracking, select the reagent lot used in the step.
   If the reagent lot is not listed, add/activate the lot on the Reagents and Controls screen.
b. Use the Preset drop-down list to help populate the fields in Step Details, as needed. For more information on presets, see *Using Step Details Presets* on page 2056. Workflow, Experiment Name, and Read 1 Cycles are required fields.
c. In the Sample Details table, enter the Final Loading Concentration. Select from the following preset options or enter a different value.
   - 225 (for PCR-free workflows)
   - 400 (for Nano workflows)
d. Select Validate Run Setup and Generate MiSeq SampleSheet.
   Clarity LIMS generates the sample sheet and attaches it and a log file to placeholders in the Files area of the Record Details screen.
e. Download the files and validate the format and content.

6. Select Next Steps.
   On the Assign Next Steps screen, samples are automatically assigned to the MiSeq Run (MiSeq v3.1) step.
7. Select Finish Step.

Using Step Details Presets

Most of the UDFs/custom fields in Step Details are analysis settings used by Clarity LIMS Product Analytics and are for advanced users only. The following fields are exceptions:

- Workflow
- Experiment Name
- Description
- Read 1 Cycles
- Read 2 Cycles
- Custom Primers

To use the default analysis settings, select the analysis module from the Preset drop-down list. The default values populate the respective fields and indicate the following actions are needed:

- Required—The field is required for the specific analysis module selected. Replace the word Required with a proper value. For example, the Genome Folder field should contain the directory to the genome folder.
- None—The field is not applicable for the specific analysis module selected. Leave the field as is.

For a list of fields that are applicable for the analysis module selected, refer to the Applicable analysis fields for the selected Workflow field in Step Details.

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Run MiSeq Run (MiSeq v3.1)

1. In Lab View, locate the MiSeq Sequencing v3.1 protocol. The pool of samples is queued for the MiSeq Run (MiSeq v3.1) step. Select the step to proceed to the Queue screen.
2. On the Queue screen, add the pool to the Ice Bucket, and then select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Record Details screen, the fields are read-only. When the run completes, the integration automatically performs the following actions:
   - Populates the fields.
   - Attaches files to the Illumina Run Report, Link to Run Folder, Run Parameters, and Run Info placeholders.
   - Populates the fields in the Sample Details table. See *MiSeq Integration v8.1 Configuration and User Interaction* on page 2019 for details.

   The Log File is attached after the next step for samples is assigned by the Next Step - Advance automation. Make sure the Illumina Run Report has been attached before continuing.
5. Select Next Steps.
   On the Assign Next Steps screen, the next step is automatically set to Mark protocol as complete.
6. Select Finish Step.

Validating Creation of Event Files

This validation checks the following information:

- The destination path is correctly configured.
- The instrument computer can access and write to the destination path.
- There are no syntax errors in the Clarity LIMS batch file.

Use the following steps to confirm that event files are created by the batch file in the destination path. The steps assume that the default configuration has been successfully imported.

1. In `C:\Illumina\gls`, double-click the batch file `gls_event_mcs_rta_lrm.bat`.
2. Confirm that an empty event file appears in the configured DESTINATION_PATH.

Manual execution of the batch file produces an output resembling the following example:

```
Filename:
event-EndRun-07295667.txt
Contents:
cycleNumber =
runFolder =
netFolder =
readType =
eventType = EndRun
softwareType = MCS
```


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```
finishDate = 2021-01-25
```

Troubleshooting

If an automation trigger does not appear to run the corresponding scripts, see the following topics:

- *Troubleshooting Automation Worker* in the *Clarity LIMS Core Functionality* documentation.
- *Troubleshooting Automation* in the *Clarity LIMS API & Database* documentation.

If an error occurs that does not provide direction on how to proceed, confirm the version of the installed MiSeq Integration Package. To confirm the version, run the `rpm -qa | grep -i miseq` command from the server console.

If the error is related to data parsing, retrieving run results data, or report values not appearing as expected, review the `MiseqIntegrator.log` file. The file is located at `/opt/gls/clarity/extensions/Illumina_MiSeq/v8/SequencingService`.

Additional troubleshooting information for this integration is provided in the *Illumina Instrument Integrations FAQ* on page 2598.

If you are unable to resolve the issue, contact the Clarity LIMS support team. Supply the relevant information from the troubleshooting already performed.

MiSeq Integration v8.1 Manual Upgrade

MiSeq Integration v8.1 includes the MiSeq v3.1 workflow upgrade. This upgrade is typically done through the API. To upgrade the workflow manually, update the validation and automation scripts as follows.

Before updating the script, make sure the MiSeq v3.0 workflow is installed.

Create Validation Scripts

Create Validation Script 1 and Validation Script 2 custom fields for the Denature and Dilute (MiSeq v3.0) master step as follows.

1. In Clarity LIMS, under Configuration, select the Custom Fields tab.
2. Under the Master Step Fields tab, select the Denature and Dilute (MiSeq v3.0) master step.
3. Using the following table, create the Validation Script 1 and Validation Script 2 custom fields.

Table 426 Custom Fields for Dilute and Denature (MiSeq v3.0) Master Step

Name	Field Type	Field Options	Additional Options
Validation Script 1	Multiline Text	• Required—Yes • Read Only—Yes	Default value provided in the drop-down section following the table. Do not activate on the step.

Name	Field Type	Field Options	Additional Options
Validation Script 2	Multiline Text	• Required—Yes • Read Only—Yes	Default value provided in the drop-down section following the table. Do not activate on the step.

Validation Script 1

```
if (step.::Experiment Name::.length() > 40) { fail(::Experiment Name cannot
exceed 40 characters.::) }; if (!step.::Experiment Name::.matches(::^(?!.*[ ]
{2})[a-zA-Z0-9-_`.~!#@%{ }]+::)) { fail(::Experiment Name contains prohibited
characters. Allowed characters are letters, numbers, periods, non-consecutive
spaces and the following special characters: `~!@#%-_}{::) }; if
(step.::Workflow:: == ::Resequencing:: && step.::Variant Caller:: ==
::Starling:: && (!step.hasValue(::Export to gVCF::) || step.::Export to gVCF::
!= ::Yes::)) { fail(::Export to gVCF must be set to Yes for Starling variant
caller.::) }; if (step.::Workflow:: == ::DNA Enrichment:: && step.::Variant
Caller:: == ::Somatic:: && (!step.hasValue(::Variant Frequency Percentage::) ||
step.::Variant Frequency Percentage:: > 100 || step.::Variant Frequency
Percentage:: < 1)) { fail(::In the Variant Frequency Percentage field, please
enter values between 1 and 100.::) }; if (step.::Workflow:: == ::DNA Amplicon::
&& step.::Variant Caller:: == ::Somatic:: && (!step.hasValue(::Variant Frequency
Percentage::) || step.::Variant Frequency Percentage:: > 30 || step.::Variant
Frequency Percentage:: < 0.05)) { fail(::In the Variant Frequency Percentage
field, please enter values between 0.05 and 30.::) }; if (!step.hasValue(::Read
2 Cycles::) || step.::Read 2 Cycles:: == 0) { if (step.::Custom
Primers::.contains(::Read 2::)) { fail(::Custom Primers setting selected is
invalid and can only be used in a Paired-End run.::) }; if (step.hasValue
(::Adapter Read 2::)) { fail(::Adapter Read 2 is only applicable for a Paired-
End run.::) }; if (step.hasValue(::UMI - Read 2 Length::) || step.hasValue(::UMI
- Read 2 Start From Cycle::)) { fail(::UMI - Read 2 Length and UMI - Read 2
Start From Cycle are only applicable for a Paired-End run.::) }; }; if
(step.hasValue(::UMI - Read 1 Length::) && !step.hasValue(::UMI - Read 1 Start
From Cycle::)) { fail(::UMI - Read 1 Start From Cycle must be greater than 0 if
UMI - Read 1 Length is greater than 0.::) }; if (!step.hasValue(::UMI - Read 1
Length::) && step.hasValue(::UMI - Read 1 Start From Cycle::)) { fail(::UMI -
Read 1 Length must be greater than 0 if UMI - Read 1 Start From Cycle is greater
than 0.::) }; if (step.hasValue(::UMI - Read 2 Length::) && !step.hasValue(::UMI
- Read 2 Start From Cycle::)) { fail(::UMI - Read 2 Start From Cycle must be
greater than 0 if UMI - Read 2 Length is greater than 0.::) }; if
(!step.hasValue(::UMI - Read 2 Length::) && step.hasValue(::UMI - Read 2 Start
From Cycle::)) { fail(::UMI - Read 2 Length must be greater than 0 if UMI - Read
```


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```
2 Start From Cycle is greater than 0.::) }; if (!step.hasValue(::UMI - Read 1
Length::) && step.hasValue(::UMI - Read 2 Length::)) { fail(::UMI - Read 1
Length must be greater than 0 if UMI - Read 2 Length is greater than 0.::) }; if
(step.hasValue(::Adapter Read 1::) && !step.::Adapter Read 1::.matches(::^
[ACGT]+(\\+[ACGT]+){0,1}$::)) { fail(::Adapter Read 1 contains prohibited
characters. Allowed characters are: ACGT+ and the adapter sequence cannot start
or end with + or contain more than one +.::) }; if (step.hasValue(::Adapter Read
2::) && !step.::Adapter Read 2::.matches(::^[ACGT]+(\\+[ACGT]+){0,1}$::)) { fail
(::Adapter Read 2 contains prohibited characters. Allowed characters are: ACGT+
and the adapter sequence cannot start or end with + or contain more than one
+.::) };
```

Validation Script 2

```
if ((step.::Aligner:: == ::BWA:: || step.::Aligner:: == ::TruSeq Amplicon
Aligner::) && step.::Workflow:: != ::DNA Amplicon::) { fail(::Aligner field
contains invalid value. Please refer to the \u005c\u0022Applicable analysis
fields for the selected Workflow:\u005c\u0022 section for more information.::)
}; if ((step.::Aligner:: == ::BWA-MEM:: || step.::Aligner:: == ::BWA-Backtrack
Legacy::) && step.::Workflow:: == ::DNA Amplicon::) { fail(::Aligner field
contains invalid value. Please refer to the \u005c\u0022Applicable analysis
fields for the selected Workflow:\u005c\u0022 section for more information.::)
}; if ((step.::Variant Caller:: == ::Starling:: || step.::Variant Caller:: ==
::GATK::) && step.::Workflow:: == ::DNA Amplicon::) { fail(::Variant Caller
field contains invalid value. Please refer to the \u005c\u0022Applicable
analysis fields for the selected Workflow:\u005c\u0022 section for more
information.::) }; if (step.::Variant Caller:: == ::Somatic:: &&
step.::Workflow:: != ::DNA Enrichment:: && step.::Workflow:: != ::DNA
Amplicon::) { fail(::Variant Caller field contains invalid value. Please refer
to the \u005c\u0022Applicable analysis fields for the selected
Workflow:\u005c\u0022 section for more information.::) }; if (step.::Variant
Caller:: == ::Germline:: && step.::Workflow:: != ::DNA Amplicon::) { fail
(::Variant Caller field contains invalid value. Please refer to the
\u005c\u0022Applicable analysis fields for the selected Workflow:\u005c\u0022
section for more information.::) }; step.::Indel-Realignment-Key:: = ::::;
step.::Indel-Realignment-Value:: = ::::; step.::Aligner-Key:: = ::::;
step.::Aligner-Value:: = ::::; step.::Variant-Caller-Value:: = ::::; if
(step.::Workflow:: == ::DNA Amplicon::) { if (step.::Indel Realignment:: ==
::Yes::) { step.::Indel-Realignment-Key:: = ::variantcallerrealignindels::;
step.::Indel-Realignment-Value:: = ::1::; }; else if (step.::Indel Realignment::
== ::No::) { step.::Indel-Realignment-Key:: = ::variantcallerrealignindels::;
step.::Indel-Realignment-Value:: = ::0::; }; if (step.::Aligner:: == ::TruSeq
```

```
Amplicon Aligner::) { step.::Aligner-Key:: = ::aligner::; step.::Aligner-Value::
= ::Amplicon::; }; else if (step.::Aligner:: == ::BWA::) { step.::Aligner-Key::
= ::aligner::; step.::Aligner-Value:: = ::BWA::; }; }; if (step.::Workflow:: !=
::DNA Amplicon::) { if (step.::Indel Realignment:: == ::Yes::) { step.::Indel-
Realignment-Key:: = ::indelrealignment::; step.::Indel-Realignment-Value:: =
::GATK::; }; else if (step.::Indel Realignment:: == ::No::) { step.::Indel-
Realignment-Key:: = ::indelrealignment::; step.::Indel-Realignment-Value:: =
::None::; }; if (step.::Aligner:: == ::BWA-Backtrack Legacy::) { step.::Aligner-
Key:: = ::runbwaaln::; step.::Aligner-Value:: = ::1::; }; else if
(step.::Aligner:: == ::BWA-MEM::) { step.::Aligner-Key:: = ::runbwaaln::;
step.::Aligner-Value:: = ::0::; }; }; if (step.hasValue(::Variant Caller::)) {
if (step.::Variant Caller:: == ::None::) { step.::Variant-Caller-Value:: = ::::;
}; if (step.::Variant Caller:: == ::GATK::) { step.::Variant-Caller-Value:: =
::GATK::; }; else if (step.::Variant Caller:: == ::Starling::) { step.::Variant-
Caller-Value:: = ::Starling::; }; else if (step.::Variant Caller:: ==
::Germline::) { step.::Variant-Caller-Value:: = ::PiscesGermline::; }; else if
(step.::Variant Caller:: == ::Somatic:: && step.::Workflow:: == ::DNA
Amplicon::) { step.::Variant-Caller-Value:: = ::PiscesSomatic::; }; else if
(step.::Variant Caller:: == ::Somatic:: && step.::Workflow:: != ::DNA
Amplicon::) { step.::Variant-Caller-Value:: = ::Somatic::; }; }; if
(step.hasValue(::Manifest::)) { step.::Manifest-Section:: = ::[Manifests]::; };
else { step.::Manifest-Section:: = ::::; }
```

4. Select Save.

Update Automation Command Lines

Update the command lines for the following automations:

- Validate Run Setup and Generate MiSeq SampleSheet
- Set Next Step - Advance

Update Validate Run Setup and Generate MiSeq SampleSheet Automation

1. From Configuration, select the Automation tab.
2. Under the Step Automation tab, select the Validate Run Setup and Generate MiSeq SampleSheet automation enabled on the Denature and Dilute (MiSeq v3.0) master step.
3. Update the command line as follows.
   Changes to the existing command line are in red. To see the previous command line, refer to the drop-down section following this step.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
```

```
script:evaluateDynamicExpression \
-exp '{udf:Validation Script 1} {udf:Validation Script 2}' \
-log {compoundOutputFileLuid3} \
-t true \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/MiSeqSamplesheetv3.csv \
-o {compoundOutputFileLuid1}.csv \
-q true \
-destLIMSID {compoundOutputFileLuid1} \
-l {compoundOutputFileLuid2}"
```

Original Command Line

The original command line script is as follows. The modified portion is in red.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-exp 'if (step.::Experiment Name::.length() > 40) { fail(::Experiment Name
cannot exceed 40 characters.::) }; if (!step.::Experiment Name::.matches(::^
(?!.*[ ]{2})[a-zA-Z0-9-_`.~!#@%{ }]+::)) { fail(::Experiment Name contains
prohibited characters. Allowed characters are letters, numbers, periods, non-
consecutive spaces and the following special characters: `~!@#%-_}{::) }; if
(step.::Workflow:: == ::Resequencing:: && step.::Variant Caller:: ==
::Starling:: && (!step.hasValue(::Export to gVCF::) || step.::Export to gVCF::
!= ::Yes::)) { fail(::Export to gVCF must be set to Yes for Starling variant
caller.::) }; if (step.::Workflow:: == ::DNA Enrichment:: && step.::Variant
Caller:: == ::Somatic:: && (!step.hasValue(::Variant Frequency Percentage::) ||
step.::Variant Frequency Percentage:: > 100 || step.::Variant Frequency
Percentage:: < 1)) { fail(::In the Variant Frequency Percentage field, please
enter values between 1 and 100.::) }; if (step.::Workflow:: == ::DNA Amplicon::
&& step.::Variant Caller:: == ::Somatic:: && (!step.hasValue(::Variant Frequency
Percentage::) || step.::Variant Frequency Percentage:: > 30 || step.::Variant
Frequency Percentage:: < 0.05)) { fail(::In the Variant Frequency Percentage
field, please enter values between 0.05 and 30.::) }; if (!step.hasValue(::Read
2 Cycles::) || step.::Read 2 Cycles:: == 0) { if (step.::Custom
Primers::.contains(::Read 2::)) { fail(::Custom Primers setting selected is
invalid and can only be used in a Paired-End run.::) }; if (step.hasValue
(::Adapter Read 2::)) { fail(::Adapter Read 2 is only applicable for a Paired-
```


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```
End run.::) }; if (step.hasValue(::UMI - Read 2 Length::) || step.hasValue(::UMI
- Read 2 Start From Cycle::)) { fail(::UMI - Read 2 Length and UMI - Read 2
Start From Cycle are only applicable for a Paired-End run.::) }; }; if
(step.hasValue(::UMI - Read 1 Length::) && !step.hasValue(::UMI - Read 1 Start
From Cycle::)) { fail(::UMI - Read 1 Start From Cycle must be greater than 0 if
UMI - Read 1 Length is greater than 0.::) }; if (!step.hasValue(::UMI - Read 1
Length::) && step.hasValue(::UMI - Read 1 Start From Cycle::)) { fail(::UMI -
Read 1 Length must be greater than 0 if UMI - Read 1 Start From Cycle is greater
than 0.::) }; if (step.hasValue(::UMI - Read 2 Length::) && !step.hasValue(::UMI
- Read 2 Start From Cycle::)) { fail(::UMI - Read 2 Start From Cycle must be
greater than 0 if UMI - Read 2 Length is greater than 0.::) }; if
(!step.hasValue(::UMI - Read 2 Length::) && step.hasValue(::UMI - Read 2 Start
From Cycle::)) { fail(::UMI - Read 2 Length must be greater than 0 if UMI - Read
2 Start From Cycle is greater than 0.::) }; if (!step.hasValue(::UMI - Read 1
Length::) && step.hasValue(::UMI - Read 2 Length::)) { fail(::UMI - Read 1
Length must be greater than 0 if UMI - Read 2 Length is greater than 0.::) }; if
(step.hasValue(::Adapter Read 1::) && !step.::Adapter Read 1::.matches(::^
[ACGT]+(\\+[ACGT]+){0,1}$::)) { fail(::Adapter Read 1 contains prohibited
characters. Allowed characters are: ACGT+ and the adapter sequence cannot start
or end with + or contain more than one +.::) }; if (step.hasValue(::Adapter Read
2::) && !step.::Adapter Read 2::.matches(::^[ACGT]+(\\+[ACGT]+){0,1}$::)) { fail
(::Adapter Read 2 contains prohibited characters. Allowed characters are: ACGT+
and the adapter sequence cannot start or end with + or contain more than one
+.::) }; if ((step.::Aligner:: == ::BWA:: || step.::Aligner:: == ::TruSeq
Amplicon Aligner::) && step.::Workflow:: != ::DNA Amplicon::) { fail(::Aligner
field contains invalid value. Please refer to the \u005c\u0022Applicable
analysis fields for the selected Workflow:\u005c\u0022 section for more
information.::) }; if ((step.::Aligner:: == ::BWA-MEM:: || step.::Aligner:: ==
::BWA-Backtrack Legacy::) && step.::Workflow:: == ::DNA Amplicon::) { fail
(::Aligner field contains invalid value. Please refer to the
\u005c\u0022Applicable analysis fields for the selected Workflow:\u005c\u0022
section for more information.::) }; if ((step.::Variant Caller:: == ::Starling::
|| step.::Variant Caller:: == ::GATK::) && step.::Workflow:: == ::DNA
Amplicon::) { fail(::Variant Caller field contains invalid value. Please refer
to the \u005c\u0022Applicable analysis fields for the selected
Workflow:\u005c\u0022 section for more information.::) }; if (step.::Variant
Caller:: == ::Somatic:: && step.::Workflow:: != ::DNA Enrichment:: &&
step.::Workflow:: != ::DNA Amplicon::) { fail(::Variant Caller field contains
invalid value. Please refer to the \u005c\u0022Applicable analysis fields for
the selected Workflow:\u005c\u0022 section for more information.::) }; if
(step.::Variant Caller:: == ::Germline:: && step.::Workflow:: != ::DNA
```

```
Amplicon::) { fail(::Variant Caller field contains invalid value. Please refer
to the \u005c\u0022Applicable analysis fields for the selected
Workflow:\u005c\u0022 section for more information.::) }; step.::Indel-
Realignment-Key:: = ::::; step.::Indel-Realignment-Value:: = ::::;
step.::Aligner-Key:: = ::::; step.::Aligner-Value:: = ::::; step.::Variant-
Caller-Value:: = ::::; if (step.::Workflow:: == ::DNA Amplicon::) { if
(step.::Indel Realignment:: == ::Yes::) { step.::Indel-Realignment-Key:: =
::variantcallerrealignindels::; step.::Indel-Realignment-Value:: = ::1::; };
else if (step.::Indel Realignment:: == ::No::) { step.::Indel-Realignment-Key::
= ::variantcallerrealignindels::; step.::Indel-Realignment-Value:: = ::0::; };
if (step.::Aligner:: == ::TruSeq Amplicon Aligner::) { step.::Aligner-Key:: =
::aligner::; step.::Aligner-Value:: = ::Amplicon::; }; else if (step.::Aligner::
== ::BWA::) { step.::Aligner-Key:: = ::aligner::; step.::Aligner-Value:: =
::BWA::; }; }; if (step.::Workflow:: != ::DNA Amplicon::) { if (step.::Indel
Realignment:: == ::Yes::) { step.::Indel-Realignment-Key:: =
::indelrealignment::; step.::Indel-Realignment-Value:: = ::GATK::; }; else if
(step.::Indel Realignment:: == ::No::) { step.::Indel-Realignment-Key:: =
::indelrealignment::; step.::Indel-Realignment-Value:: = ::None::; }; if
(step.::Aligner:: == ::BWA-Backtrack Legacy::) { step.::Aligner-Key:: =
::runbwaaln::; step.::Aligner-Value:: = ::1::; }; else if (step.::Aligner:: ==
::BWA-MEM::) { step.::Aligner-Key:: = ::runbwaaln::; step.::Aligner-Value:: =
::0::; }; }; if (step.hasValue(::Variant Caller::)) { if (step.::Variant
Caller:: == ::None::) { step.::Variant-Caller-Value:: = ::::; }; if
(step.::Variant Caller:: == ::GATK::) { step.::Variant-Caller-Value:: =
::GATK::; }; else if (step.::Variant Caller:: == ::Starling::) { step.::Variant-
Caller-Value:: = ::Starling::; }; else if (step.::Variant Caller:: ==
::Germline::) { step.::Variant-Caller-Value:: = ::PiscesGermline::; }; else if
(step.::Variant Caller:: == ::Somatic:: && step.::Workflow:: == ::DNA
Amplicon::) { step.::Variant-Caller-Value:: = ::PiscesSomatic::; }; else if
(step.::Variant Caller:: == ::Somatic:: && step.::Workflow:: != ::DNA
Amplicon::) { step.::Variant-Caller-Value:: = ::Somatic::; }; }; if
(step.hasValue(::Manifest::)) { step.::Manifest-Section:: = ::[Manifests]::; };
else { step.::Manifest-Section:: = ::::; }' \
-log {compoundOutputFileLuid3} \
-t true \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/MiSeqSamplesheetv3.csv \
-o {compoundOutputFileLuid1}.csv \
```

```
-q true \
-destLIMSID {compoundOutputFileLuid1} \
-l {compoundOutputFileLuid2}"
```

4. Select Save.

Update Set Next Step - Advance Automation

1. From Configuration, select the Automation tab.
2. Under the Step Automation tab, select the Set Next Step - Advance automation enabled on the Denature and Dilute (MiSeq v3.0) master step.
3. Update the command line as follows. To see the previous command line, refer to the drop-down section following this step.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -u {username} -p {password} \
script:evaluateDynamicExpression \
-i {stepURI:v2} \
-t false \
-h false \
-exp 'nextStep = ::ADVANCE::' \
-log {compoundOutputFileLuid3}"
```

Original Command Line

The original command line script is as follows. The deleted portion is in red.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -u {username} -p {password} \
script:setUDF \
-i {processURI:v2} \
-f 'Progress' \
-t '//input/@uri->//sample/@uri' \
-v 'Library ready for sequencing' \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -u {username} -p {password} \
script:evaluateDynamicExpression \
-i {stepURI:v2} \
-t false \
-h false \
-exp 'nextStep = ::ADVANCE::' \
-log {compoundOutputFileLuid3}"
```


For Research Use Only. Not for use in diagnostic procedures.

4. Select Save.

MiSeq Integration v8.1 Release Notes

These Release Notes describe the key changes to software components for the Clarity LIMS MiSeq Integration Package since version 8.0.

This is an optional software update for customers interested in the new features available with this release.

If upgrading from a version prior to version 8.0, review the release notes for v8.0 for a list of features and bug fixes introduced in that version.

Compatibility

This version of the integration package is compatible with the following software:

- Clarity LIMS v5.1 and later
- NGS Extensions Package v5.21 and later
- MiSeq Control Software (MCS) v4.0
- Local Run Manager v3.0

New Features

Features provided with this release of the integration package are as follows.

- Support for Clarity LIMS v6.0. Clarity LIMS v4.x and v5.0 are not supported.
- Tech refresh to upgrade third party libraries.
- Fixes to address the following Apache log4j vulnerabilities:
  - CVE-2021-44228
  - CVE-2021-45046
  - CVE-2021-45105
  - CVE-2021-44832

Defects Fixed

The MiSeq sequencing service now records interopUtil parsing in the log file.

Known Issues

None

MiSeq Integration v8.0 Configuration and User Interaction

The Illumina MiSeq Integration Package v8.0 supports the integration of Clarity LIMS to Illumina MiSeq Sequencing Systems.

The integration allows for automated tracking of an Illumina sequencing run in Clarity LIMS. This capability includes tracking sequencing run status, generating run report, and capturing and parsing run statistics. In addition, this integration provides automated generation of a sample sheet file for use with the MiSeq Control Software (MCS) and Local Run Manager.

Overview

The Illumina MiSeq Integration Package v8.0 includes the following features:

- Preconfigured MiSeq Sequencing v3.0 workflow that maps to lab protocol steps and sequencing runs.
- Automated generation of a sample sheet for use with the MiSeq Control Software (MCS) and Local Run Manager.
- Supported Local Run Manager analysis modules are GenerateFastQ, Library QC, Resequencing, DNA Enrichment, and DNA Amplicon.
- Automated tracking of the MiSeq sequencing run and parsing of run statistics into Clarity LIMS, including:
  - Run status and metrics of sequencing run
  - Sequencing run parameters
  - Real-Time Analysis (RTA) v1 run directory location and other run-specific information

This integration also includes a preconfigured Library Prep Validation workflow that is used for validation purposes only. The workflow contains a single-step protocol that models the library prep required to produce normalized libraries that are ready for the MiSeq Sequencing v3.0 workflow. For details, see *MiSeq Integration v8.0 Validation and Troubleshooting* on page 2100.

Depending on the Clarity LIMS version, install the workflows as follows.

- For Clarity LIMS v4 (4.2 and later), install the following workflows via Preconfigured Workflows Package (PCWP) v2.1:
  - MiSeq Sequencing v3.0 workflow
  - Library Prep Validation v1.1.2 workflow
- For Clarity LIMS v5 (5.1 and later), install the following workflows via Illumina Preset Protocols (IPP) v2.1:
  - MiSeq Sequencing v3.0 workflow
  - Library Prep Validation v2.0.2 workflow

The following sections describe the MiSeq Sequencing v3.0 workflow.

For instructions on validating and troubleshooting the Illumina MiSeq Integration Package, see *MiSeq Integration v8.0 Validation and Troubleshooting* on page 2100.

Compatibility

MiSeq Integration v8.0 is not supported on Clarity LIMS v5.0.

MiSeq Integration v8.0 is only required for MCS (MiSeq Control Software) v4.0. For users on MCS v3.1 and earlier, refrain from upgrading to MiSeq Integration v8.0 as it breaks the integration.

Prerequisites and Assumptions

MiSeq Sequencing v3.0 workflow is only compatible with MiSeq INT v8.0 package.

Before samples are assigned to the workflow, make sure that the following prerequisites are completed.

- Samples have been run through QC and library prep.
- Samples have been normalized, and the value is captured in a field called Normalized Molarity (nM).
- Reagent labels are attached to samples.
- Samples have been accessioned into Clarity LIMS.

For more information on sample accessioning, see *Sample Accessioning* and *Upload and Modify Samples* in the Clarity LIMS Core Functionality documentation.

Samples can be assigned to the MiSeq Sequencing v3.0 workflow automatically using a routing script or manually from the Projects & Samples dashboard. See *Assign and Process Samples* in the Clarity LIMS Core Functionality documentation.

Workflows, Protocols, and Steps

The Illumina MiSeq Integration includes the MiSeq Sequencing v3.0 workflow, which contains a single protocol of the same name.

The protocol includes the following steps and automations.

Table 427 MiSeq Sequencing v3.0 Protocol Steps and Automations

Step	Automation
1. Library Pooling (MiSeq v3.0)	• Validate Maximum Number of Samples • Set Next Step - Advance
2. Denature and Dilute (MiSeq v3.0)	• Validate Run Setup and Generate MiSeq SampleSheet • Set Next Step - Advance
3. MiSeq Run (MiSeq v3.0)	• Set Next Step - Advance

Terminology

This integration supports Clarity LIMS v4.2 and later.

As of Clarity LIMS v5, the term user-defined field (UDF) has been replaced with custom field in the user interface. However, the API resource is still called UDF.

There are two types of custom fields:

- Master step fields—Configured on master steps. Master step fields only apply to the following:
  - The master step on which the fields are configured.
  - The steps derived from those master steps.
- Global fields—Configured on entities (eg, submitted sample, derived sample, measurement, etc.). Global fields apply to the entire Clarity LIMS system.

Step 1: Library Pooling (MiSeq v3.0)

In this step, the lab scientist manually places libraries into pools in the Clarity LIMS Placement screen.

Set Next Step - Advance Automation

This automation advances samples to the next step in the protocol. The automation is automatically triggered on exit of the Record Details screen.

Default command line:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-t false \
-h false \
-exp 'nextStep = ::ADVANCE::' \
-log {compoundOutputFileLuid0}"
```

Validate Maximum Number of Samples Automation

This automation checks the maximum number of samples that is allowed in a single Pool. The default maximum for Illumina MiSeq Control Software is 1536 samples. The automation is automatically triggered on entry of the Pooling screen.

Default command line:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:validateSampleCount -min 1 -max 1536"
```

Step UDFs/Custom Fields

One field is configured on the Library Pooling (MiSeq v3.0) step. The field displays on the Record Details screen at run time.

Table 428 Library Pooling (MiSeq v3.0) Step UDF/Custom Field Configuration

Field Name	Field Type	Field Constraints/Options
Comment	Multiline Text	• Required: No • Read Only: No

Global UDFs/Custom Fields

The following table lists the global UDFs/fields that are displayed on the Queue and Ice Bucket screens of the Library Pooling (MiSeq v3.0) step. Most fields display in expanded view only.

Table 429 Global UDF/Custom Field Configuration for Library Pooling (MiSeq v3.0)

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Configured on Sample in Clarity LIMS v4 and Submitted Sample in Clarity LIMS v5:			

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Application	Single-line Text (v4) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: Yes	• TruSeq mRNA sequencing • TruSeq DNA sequencing (large genome *de novo*) • TruSeq DNA sequencing (large genome reseq) • TruSeq DNA sequencing (small genome *de novo*) • TruSeq DNA sequencing (small genome reseq) • Nextera DNA sequencing • TruSeq Custom Amplicon sequencing • ChIP-sequencing • Exome sequencing • Mate pair sequencing • Small RNA sequencing
Pooling	Single-line Text (v4) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: Yes	• Yes • No
Read Length	Single-line Text (v4) Text (v5)	• Required: No • Read Only: No	Not applicable
Sequencing Coverage	Single-line Text (v4) Text (v5)	• Required: No • Read Only: No	Not applicable

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Sequencing Method	Single-line Text (v4) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: Yes	• Single Read • Paired End Read • Indexed Single Read • Indexed Paired End Read
Configured on Analyte in Clarity LIMS v4 and Derived Sample in Clarity LIMS v5:			
Normalized Molarity (nM)	Numeric	• Required: No • Read Only: No	Decimal Places Displayed: 2

User Interaction

1. On the Queue screen, do as follows.
   a. Add the samples to the Ice Bucket.
   b. [Optional] In the Add Control Samples panel, add the PhiX v3 control sample to the Ice Bucket.
   c. Select View Ice Bucket.
2. On the Ice Bucket screen, select Begin Work.
   The Validate Maximum Number of Samples automation script runs. The maximum number of samples allowed is 1536.
3. On the Pool Samples screen, do as follows.
   a. Create a pool of samples by dragging samples into the Pool Creator.
   b. Name the pool or accept the default name (Pool #1).
   c. Select Place Samples.
4. On the Placement screen, do as follows.
   a. Select the pool in the Samples to be Placed area and drag it to the container.
   b. Select Record Details.
5. On the Record Details screen, select Next Steps.
   On the Assign Next Steps screen, next step is already set to Denature and Dilute (MiSeq v3.0).
6. Select Finish Step.

Step 2: Denature and Dilute (MiSeq v3.0)

In this step, pooled libraries are denatured and diluted, and then placed into the reagent cartridge loaded into the MiSeq instrument.

Validate Run Setup and Generate MiSeq SampleSheet Automation

This automation validates the information entered on the Record Details screen, generates the sample sheet, and attaches the sample sheet to the Denature and Dilute step. Select a button on the Record Details screen to trigger this automation. For details, see *Sample Sheet Generation* on page 2092.

Validation Rules:

- The Experiment Name UDF cannot exceed 40 characters.
- The Experiment Name UDF can only contain letters, numbers, periods, nonconsecutive spaces, and the following special characters: `~!@#%-_}{.
- If Starling is selected as the variant caller, the Export to gVCF UDF must be set to Yes.
- If the Read 2 Cycles UDF is empty or 0, the following rules apply:
  - The Custom Primers UDF cannot have a selection that contains Read 2.
  - The Adapter Read 2 UDF cannot have a value.
  - The UMI - Read 2 Length UDF and the UMI - Read 2 Start From Cycle UDF cannot have values.
- The UMI - Read 1 Length UDF and the UMI - Read 1 Start From Cycle UDF cannot be used independently.
- The UMI - Read 2 Length UDF and the UMI - Read 2 Start From Cycle UDF cannot be used independently.
- If the UMI - Read 2 Length UDF is greater than 0, the UMI - Read 1 Length UDF must be greater than 0.
- The Adapter Read 1 and Adapter Read 2 UDFs can only contain ACGT+ characters. The adapter sequence cannot start or end with + or contain more than one +.
- For DNA Enrichment analysis, if the Variant Caller value is Somatic, the Variant Frequency Percentage must be between 1 and 100.
- For DNA Amplicon analysis, if the Variant Caller value is Somatic, the Variant Frequency Percentage field must contain a value between 0.05 and 30.
- If the Aligner value is BWA or TruSeq Amplicon Aligner, an error occurs when the Workflow value is not equal to DNA Amplicon.
- If the Aligner value is BWA-MEM or BWA-Backtrack Legacy, an error occurs when the Workflow value is equal to DNA Amplicon.
- If the Variant Caller value is Starling or GATK, an error occurs when the Workflow value is equal to DNA Amplicon.
- If the Variant Caller value is Somatic, an error occurs when the Workflow value is not equal to DNA Enrichment or DNA Amplicon.
- If the Variant Caller value is Germline, an error occurs when the Workflow value is not equal to DNA Amplicon.

For Research Use Only. Not for use in diagnostic procedures.

Default command line (Clarity LIMS v4 and v5):

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-exp 'if (step.::Experiment Name::.length() > 40) { fail(::Experiment Name
cannot exceed 40 characters.::) }; if (!step.::Experiment Name::.matches(::^
(?!.*[ ]{2})[a-zA-Z0-9-_`.~!#@%{ }]+::)) { fail(::Experiment Name contains
prohibited characters. Allowed characters are letters, numbers, periods, non-
consecutive spaces and the following special characters: `~!@#%-_}{::) }; if
(step.::Workflow:: == ::Resequencing:: && step.::Variant Caller:: ==
::Starling:: && (!step.hasValue(::Export to gVCF::) || step.::Export to gVCF::
!= ::Yes::)) { fail(::Export to gVCF must be set to Yes for Starling variant
caller.::) }; if (step.::Workflow:: == ::DNA Enrichment:: && step.::Variant
Caller:: == ::Somatic:: && (!step.hasValue(::Variant Frequency Percentage::) ||
step.::Variant Frequency Percentage:: > 100 || step.::Variant Frequency
Percentage:: < 1)) { fail(::In the Variant Frequency Percentage field, please
enter values between 1 and 100.::) }; if (step.::Workflow:: == ::DNA Amplicon::
&& step.::Variant Caller:: == ::Somatic:: && (!step.hasValue(::Variant Frequency
Percentage::) || step.::Variant Frequency Percentage:: > 30 || step.::Variant
Frequency Percentage:: < 0.05)) { fail(::In the Variant Frequency Percentage
field, please enter values between 0.05 and 30.::) }; if (!step.hasValue(::Read
2 Cycles::) || step.::Read 2 Cycles:: == 0) { if (step.::Custom
Primers::.contains(::Read 2::)) { fail(::Custom Primers setting selected is
invalid and can only be used in a Paired-End run.::) }; if (step.hasValue
(::Adapter Read 2::)) { fail(::Adapter Read 2 is only applicable for a Paired-
End run.::) }; if (step.hasValue(::UMI - Read 2 Length::) || step.hasValue(::UMI
- Read 2 Start From Cycle::)) { fail(::UMI - Read 2 Length and UMI - Read 2
Start From Cycle are only applicable for a Paired-End run.::) }; }; if
(step.hasValue(::UMI - Read 1 Length::) && !step.hasValue(::UMI - Read 1 Start
From Cycle::)) { fail(::UMI - Read 1 Start From Cycle must be greater than 0 if
UMI - Read 1 Length is greater than 0.::) }; if (!step.hasValue(::UMI - Read 1
Length::) && step.hasValue(::UMI - Read 1 Start From Cycle::)) { fail(::UMI -
Read 1 Length must be greater than 0 if UMI - Read 1 Start From Cycle is greater
than 0.::) }; if (step.hasValue(::UMI - Read 2 Length::) && !step.hasValue(::UMI
- Read 2 Start From Cycle::)) { fail(::UMI - Read 2 Start From Cycle must be
greater than 0 if UMI - Read 2 Length is greater than 0.::) }; if
(!step.hasValue(::UMI - Read 2 Length::) && step.hasValue(::UMI - Read 2 Start
From Cycle::)) { fail(::UMI - Read 2 Length must be greater than 0 if UMI - Read
2 Start From Cycle is greater than 0.::) }; if (!step.hasValue(::UMI - Read 1
Length::) && step.hasValue(::UMI - Read 2 Length::)) { fail(::UMI - Read 1
Length must be greater than 0 if UMI - Read 2 Length is greater than 0.::) }; if
```

```
(step.hasValue(::Adapter Read 1::) && !step.::Adapter Read 1::.matches(::^
[ACGT]+(\\+[ACGT]+){0,1}$::)) { fail(::Adapter Read 1 contains prohibited
characters. Allowed characters are: ACGT+ and the adapter sequence cannot start
or end with + or contain more than one +.::) }; if (step.hasValue(::Adapter Read
2::) && !step.::Adapter Read 2::.matches(::^[ACGT]+(\\+[ACGT]+){0,1}$::)) { fail
(::Adapter Read 2 contains prohibited characters. Allowed characters are: ACGT+
and the adapter sequence cannot start or end with + or contain more than one
+.::) }; if ((step.::Aligner:: == ::BWA:: || step.::Aligner:: == ::TruSeq
Amplicon Aligner::) && step.::Workflow:: != ::DNA Amplicon::) { fail(::Aligner
field contains invalid value. Please refer to the \u005c\u0022Applicable
analysis fields for the selected Workflow:\u005c\u0022 section for more
information.::) }; if ((step.::Aligner:: == ::BWA-MEM:: || step.::Aligner:: ==
::BWA-Backtrack Legacy::) && step.::Workflow:: == ::DNA Amplicon::) { fail
(::Aligner field contains invalid value. Please refer to the
\u005c\u0022Applicable analysis fields for the selected Workflow:\u005c\u0022
section for more information.::) }; if ((step.::Variant Caller:: == ::Starling::
|| step.::Variant Caller:: == ::GATK::) && step.::Workflow:: == ::DNA
Amplicon::) { fail(::Variant Caller field contains invalid value. Please refer
to the \u005c\u0022Applicable analysis fields for the selected
Workflow:\u005c\u0022 section for more information.::) }; if (step.::Variant
Caller:: == ::Somatic:: && step.::Workflow:: != ::DNA Enrichment:: &&
step.::Workflow:: != ::DNA Amplicon::) { fail(::Variant Caller field contains
invalid value. Please refer to the \u005c\u0022Applicable analysis fields for
the selected Workflow:\u005c\u0022 section for more information.::) }; if
(step.::Variant Caller:: == ::Germline:: && step.::Workflow:: != ::DNA
Amplicon::) { fail(::Variant Caller field contains invalid value. Please refer
to the \u005c\u0022Applicable analysis fields for the selected
Workflow:\u005c\u0022 section for more information.::) }; step.::Indel-
Realignment-Key:: = ::::; step.::Indel-Realignment-Value:: = ::::;
step.::Aligner-Key:: = ::::; step.::Aligner-Value:: = ::::; step.::Variant-
Caller-Value:: = ::::; if (step.::Workflow:: == ::DNA Amplicon::) { if
(step.::Indel Realignment:: == ::Yes::) { step.::Indel-Realignment-Key:: =
::variantcallerrealignindels::; step.::Indel-Realignment-Value:: = ::1::; };
else if (step.::Indel Realignment:: == ::No::) { step.::Indel-Realignment-Key::
= ::variantcallerrealignindels::; step.::Indel-Realignment-Value:: = ::0::; };
if (step.::Aligner:: == ::TruSeq Amplicon Aligner::) { step.::Aligner-Key:: =
::aligner::; step.::Aligner-Value:: = ::Amplicon::; }; else if (step.::Aligner::
== ::BWA::) { step.::Aligner-Key:: = ::aligner::; step.::Aligner-Value:: =
::BWA::; }; }; if (step.::Workflow:: != ::DNA Amplicon::) { if (step.::Indel
Realignment:: == ::Yes::) { step.::Indel-Realignment-Key:: =
::indelrealignment::; step.::Indel-Realignment-Value:: = ::GATK::; }; else if
```

```
(step.::Indel Realignment:: == ::No::) { step.::Indel-Realignment-Key:: =
::indelrealignment::; step.::Indel-Realignment-Value:: = ::None::; }; if
(step.::Aligner:: == ::BWA-Backtrack Legacy::) { step.::Aligner-Key:: =
::runbwaaln::; step.::Aligner-Value:: = ::1::; }; else if (step.::Aligner:: ==
::BWA-MEM::) { step.::Aligner-Key:: = ::runbwaaln::; step.::Aligner-Value:: =
::0::; }; }; if (step.hasValue(::Variant Caller::)) { if (step.::Variant
Caller:: == ::None::) { step.::Variant-Caller-Value:: = ::::; }; if
(step.::Variant Caller:: == ::GATK::) { step.::Variant-Caller-Value:: =
::GATK::; }; else if (step.::Variant Caller:: == ::Starling::) { step.::Variant-
Caller-Value:: = ::Starling::; }; else if (step.::Variant Caller:: ==
::Germline::) { step.::Variant-Caller-Value:: = ::PiscesGermline::; }; else if
(step.::Variant Caller:: == ::Somatic:: && step.::Workflow:: == ::DNA
Amplicon::) { step.::Variant-Caller-Value:: = ::PiscesSomatic::; }; else if
(step.::Variant Caller:: == ::Somatic:: && step.::Workflow:: != ::DNA
Amplicon::) { step.::Variant-Caller-Value:: = ::Somatic::; }; }; if
(step.hasValue(::Manifest::)) { step.::Manifest-Section:: = ::[Manifests]::; };
else { step.::Manifest-Section:: = ::::; }' \
-log {compoundOutputFileLuid3} \
-t true \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p
{password} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/MiSeqSamplesheetv3.csv \
-o {compoundOutputFileLuid1}.csv \
-q true \
-destLIMSID {compoundOutputFileLuid1} \
-l {compoundOutputFileLuid2}"
```

Set Next Step - Advance Automation

This automation advances samples to the next step in the protocol. The automation is automatically triggered by exiting the Record Details screen.

In Clarity LIMS v4, the automation also invokes the setUDF script, which sets the value of the Progress UDF. Clarity LIMS updates this UDF value as samples progress through the workflow steps. The Progress UDF does not display in the Clarity LIMS user interface but does display in the LabLink interface. This feature allows collaborators to see the status of samples.

Default command line (Clarity LIMS v4):

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -u {username} -p {password} \
```

```
script:setUDF \
-i {processURI:v2} \
-f 'Progress' \
-t '//input/@uri->//sample/@uri' \
-v 'Library ready for sequencing' \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -u {username} -p {password} \
script:evaluateDynamicExpression \
-i {stepURI:v2} \
-t false \
-h false \
-exp 'nextStep = ::ADVANCE::' \
-log {compoundOutputFileLuid3}"
```

Default command line (Clarity LIMS v5):

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-t false \
-h false \
-exp 'nextStep = ::ADVANCE::' \
-log {compoundOutputFileLuid3}"
```

Step UDFs/Custom Fields

Most fields configured on the Denature and Dilute (MiSeq v3.0) step display on the Record Details screen in the Step Data table.

These fields are manually populated at run time (see *User Interaction* on page 2088). The values are then used to generate the sample sheet. For details on the sample generation functionality, see *Sample Sheet Generation* on page 2092.

Table 430 Denature & Dilute (MiSeq v2.0) Step UDF/Custom Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Adapter Read 1	Single-line Text (v4) Text (v5)	• Required: No • Read Only: No	Not applicable

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Adapter Read 2	Single-line Text (v4) Text (v5)	• Required: No • Read Only: No	Not applicable
Aligner	Single-line Text (v4) Text (v5)	• Required: No • Read Only: No • Custom Entries: No	• None (default) • BWA-MEM • BWA-Backtrack Legacy • BWA • TruSeq Amplicon Aligner
Aligner-Key*	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes • Custom Entries: No	Not applicable
Aligner-Value*	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes • Custom Entries: No	Not applicable
Annotation	Single-line Text (v4) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: No	• None • RefSeq • Ensembl
Applicable analysis fields for the selected Workflow	Multiline Text	• Required: No • Read Only: Yes	Select a value from the drop-down list in the upper-right corner.
Comment	Multiline Text	• Required: No • Read Only: No	Not applicable
Custom Primers	Single-line Text (v4) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: No	• None (Default) • Read 1 • Index • Read 2 • Read 1, Index • Read 1, Index, Read 2 • Read 1, Read 2 • Index, Read 2

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Description	Single-line Text (v4) Text (v5)	• Required: No • Read Only: No	Not applicable
Experiment Name	Single-line Text (v4) Text (v5)	• Required: Yes • Read Only: No	Not applicable
Export to gVCF	Single-line Text (v4) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: No	• None (default) • Yes • No
Flag PCR Duplicates	Single-line Text (v4) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: No	• None (default) • Yes • No
Genome Folder	Single-line Text (v4) Text (v5)	• Required: No • Read Only: No	Not applicable
Indel Realignment	Single-line Text (v4) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: No	• None (default) • Yes • No
Indel Repeat Filter Cutoff	Single-line Text (v4) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: No	• None (default) • Yes • No
Indel-Realignment-Key*	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes • Custom Entries: No	Not applicable
Indel-Realignment-Value*	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes • Custom Entries: No	Not applicable
Manifest	Single-line Text (v4) Text (v5)	• Required: No • Read Only: No	Not applicable
Manifest Padding	Single-line Text (v4) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: No	• None (default) • 0 • 50 • 100 • 150 • 200 • 250

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Manifest-Section*	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes • Custom Entries: No	Not applicable
Picard HS Metric Reporting	Single-line Text (v4) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: No	• None (default) • Yes • No
Read 1 Cycles	Numeric (v4) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 251 (Default) • 151 • 101 • 76 • 51 • Range From: 1 to 1000 • Decimal Places Displayed: 0
Read 2 Cycles	Numeric (v4) Numeric Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: Yes	• 251 • 151 • 101 • 76 • 51 • Range From: 0 to 1000 • Decimal Places Displayed: 0
Read Stitching	Single-line Text (v4) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: No	• None (default) • Yes • No
Reverse Complement	Single-line Text (v4) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: No	• None (default) • Yes • No
UMI - Read 1 Length	Numeric	• Required: No • Read Only: No	• Minimum value: 1
UMI - Read 1 Start From Cycle	Numeric	• Required: No • Read Only: No	• Minimum value: 1

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
UMI - Read 2 Length	Numeric	• Required: No • Read Only: No	• Minimum value: 1
UMI - Read 2 Start From Cycle	Numeric	• Required: No • Read Only: No	• Minimum value: 1
Variant Caller	Single-line Text (v4) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: No	• None (Default) • GATK • Germline • Somatic • Starling
Variant Caller Depth Filter	Numeric	• Required: No • Read Only: No	• Minimum value: 10 • Maximum value: 10000
Variant Quality Filter	Numeric	• Required: No • Read Only: No	• Minimum value: 2 • Maximum value: 1000
Variant Frequency Percentage	Numeric	• Required: No • Read Only: No	• Minimum value: 0.05 • Maximum value: 100
Variant-Caller-Value*	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes • Custom Entries: No	Not applicable
Workflow	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• GenerateFASTQ • LibraryQC • Resequencing • DNA Enrichment • DNA Amplicon

*Not displayed on the Record Details screen at run time. Used for sample sheet generation only.

The following 6 UDFs are hidden and read-only UDFs. The only purpose of these UDFs is to aid sample sheet generation.

Do not change these UDFs, doing so can cause sample sheet generation not to work properly.

- Aligner-Key

- Aligner-Value
- Indel-Realignment-Key
- Indel-Realignment-Value
- Manifest-Section
- Variant-Caller-Value

Groups of Defaults/Presets

The following groups of defaults/presets are configured for Denature and Dilute (MiSeq v3.0) step UDFs/master step custom fields:

Resequencing

Field	Configuration
Workflow	Resequencing
Read 1 Cycles	251
Custom Primers	None
Aligner	BWA-MEM
Annotation	None
Export to gVCF	No
Flag PCR Duplicates	Yes
Genome Folder	Required
Indel Realignment	No
Indel Repeat Filter Cutoff	None
Manifest	None
Manifest Padding	None
Picard HS Metric Reporting	None
Reverse Complement	None
Read Stitching	None
Variant Caller	GATK

Field	Configuration
Workflow	Resequencing
Read 1 Cycles	251
Applicable analysis fields for the selected Workflow	• Aligner (BWA-Backtrack Legacy, BWA-MEM) • Export to gVCF • Flag PCR Duplicates • Genome Folder (fill in the folder path) • Indel Realignment • Variant Caller (Starling, GATK)

Library QC

Field	Configuration
Workflow	LibraryQC
Read 1 Cycles	251
Custom Primers	None
Aligner	None
Annotation	None
Export to gVCF	None
Flag PCR Duplicates	Yes
Genome Folder	Required
Indel Realignment:	None
Indel Repeat Filter Cutoff	None
Manifest	None
Manifest Padding	None
Picard HS Metric Reporting	None
Reverse Complement	None
Read Stitching	None
Variant Caller	None
Applicable analysis fields for the selected Workflow	• Flag PCR Duplicates • Genome Folder (fill in the folder path)

GenerateFastQ

Field	Configuration
Workflow	GenerateFastQ
Read 1 Cycles	251
Custom Primers	None
Aligner	None
Annotation	None
Export to gVCF	None
Flag PCR Duplicates	None
Genome Folder	None
Indel Realignment	None
Indel Repeat Filter Cutoff	None
Manifest	None
Manifest Padding	None
Picard HS Metric Reporting	None
Reverse Complement	None
Read Stitching	None
Variant Caller	None
Applicable analysis fields for the selected Workflow	None

DNA Enrichment

Field	Configuration
Workflow	DNA Enrichment
Read 1 Cycles	251
Custom Primers	None
Aligner	BWA-MEM
Annotation	None
Export to gVCF	None
Flag PCR Duplicates	Yes

Field	Configuration
Genome Folder	Required
Indel Realignment	Yes
Indel Repeat Filter Cutoff	None
Manifest	Required
Manifest Padding	150
Picard HS Metric Reporting	No
Reverse Complement	None
Read Stitching	None
Variant Caller	Starling
Applicable analysis fields for the selected Workflow	• Aligner (BWA-Backtrack Legacy, BWA-MEM) • Flag PCR Duplicates • Genome Folder (fill in the folder path) • Indel Realignment • Indel Repeat Filter Cutoff (if Variant Caller is Somatic) • Manifest (fill in the file name) • Manifest Padding • Picard HS Metric Reporting • Variant Caller (Starling, Somatic, GATK) • Variant Frequency Percentage (if Variant Caller is Somatic)

DNA Amplicon

Field	Configuration
Workflow	DNA Amplicon
Read 1 Cycles	251
Custom Primers	None

Field	Configuration
Aligner	BWA
Annotation	RefSeq
Export to gVCF	None
Flag PCR Duplicates	None
Genome Folder	Required
Indel Realignment	Yes
Indel Repeat Filter Cutoff	None
Manifest	Required
Manifest Padding	None
Picard HS Metric Reporting	None
Reverse Complement	None
Read Stitching	None
Variant Caller	Germline
Variant Caller Depth Filter	10
Variant Quality Filter	30
Applicable analysis fields for the selected Workflow	• Aligner (BWA, TruSeq Amplicon Aligner) • Annotation (RefSeq, Ensembl) • Genome Folder (fill in the folder path) • Indel Realignment • Manifest (fill in the file name) • Read stitching (if Aligner is TruSeq Amplicon Aligner) • Variant Caller (Germline, Somatic) • Variant Caller Depth Filter (10–10000) • Variant Frequency Percentage (if Variant Caller is Somatic) • Variant Quality Filter (2–1000)

Global UDFs/Custom Fields

The following table shows the global UDFs/fields that are configured to display on the Queue, Ice Bucket, and Record Details screens of the Denature and Dilute (MiSeq v3.0) step.

Table 431 Global UDF/Custom Field Configuration for Denature and Dilute (MiSeq v3.0) Step

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Configured on Sample in LIMS v4 and Submitted Sample in LIMS v5:			
Progress *(LIMS v4) Displays in LabLink Collaborations Interface only*	Single-line Text (v4) Text (v5)	• Required: No • Read Only: No	Not applicable
Read Length *Displays on Queue & Ice Bucket screens*	Single-line Text (v4) Text (v5)	• Required: No • Read Only: No	Not applicable
Sequencing Method *Displays on Queue & Ice Bucket screens*	Single-line Text (v4) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: Yes	• Single Read • Paired End Read • Indexed Single Read • Indexed Paired End Read
Configured on Analyte in LIMS v4 and Derived Sample in LIMS v5:			
Final Loading Concentration *Displays on Record Details screen*	Numeric (v4) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 225 • 400

Step File Placeholders

Placeholders for the following files are configured on the Record Details screen of the Denature and Dilute (MiSeq v3.0) step:

- Lab Tracking Form (manually generated)
- MiSeq SampleSheet (autogenerated)
- MiSeq SampleSheet Generation Log (autogenerated)
- Log File (autogenerated)

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Table 432 Sample Sheet Files and Placeholders Generated by Denature and Dilute (MiSeq v3.0) Step

Item	Description
Lab Tracking Form placeholder	This placeholder in Clarity LIMS allows the lab scientist to attach a lab-specific tracking form to the step manually.
MiSeq SampleSheet	This CSV file is automatically generated by Clarity LIMS for use with the MiSeq system. The file can be opened as a text file or an Excel spreadsheet.
MiSeq SampleSheet Generation Log	This log file is automatically generated by Clarity LIMS. The log file captures any errors that Clarity LIMS might encounter when generating the sample sheet.
Log File	This log file is automatically generated by Clarity LIMS. The log file captures the status of the EvaluateDynamicExpression script that is invoked by the Set Next Step - Advance automation.

User Interaction

1. On the Queue screen, add the pool to the Ice Bucket, and then select View Ice Bucket.
2. On the Ice Bucket screen, select Begin Work.
3. On the Placement screen, do as follows.
   a. Scan the barcode of the MiSeq reagent cartridge into the MiSeq Reagent Cartridge field.
   b. Place the pool of samples into the reagent cartridge.
   c. Select Record Details.
4. On the Record Details screen, do as follows.

a. Under Reagent Lot Tracking, select the reagent lot used in the step.
If the reagent lot is not listed, add/activate the lot on the Reagents and Controls screen.

b. Populate the fields in the Step Details table, as needed. Workflow, Experiment Name, and Read 1 Cycles are required fields. Refer to *MiSeq Integration v8.0 Validation and Troubleshooting* on page 2100 for details on how to autopopulate the UDFs by using the preset drop-down list.

c. In the Sample Details table, enter the Final Loading Concentration. Select from the following preset options or enter a different value.
   - 225 (for PCR-free workflows)
   - 400 (for Nano workflows)

d. Select Validate Run Setup and Generate MiSeq SampleSheet.
Clarity LIMS generates the sample sheet and attaches it and a log file to placeholders in the Files area of the Record Details screen.

e. Download the files and validate their format and content.

5. Select Next Steps.
On the Assign Next Steps screen, samples are already assigned to the MiSeq Run (MiSeq v3.0) step.
6. Select Finish Step.

See also *Sample Sheet Generation* on page 2092.

Step 3: MiSeq Run (MiSeq v3.0)

Set Next Step - Advance Automation

This automation advances samples to the next step in the protocol. The automation is automatically triggered by exiting the Record Details screen.

Default command line:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-t false \
-h false \
-exp 'nextStep = ::ADVANCE::' \
-log {compoundOutputFileLuid5}"
```

Step UDFs/Custom Fields

There are 18 fields configured on the MiSeq Run (MiSeq v3.0) step. These fields display on the Record Details screen at run time.

The read-only fields are automatically populated at the end of the run.

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Table 433 MiSeq Run (MiSeq v3.0) Step UDF/Custom Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Chemistry	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable
Comment	Multiline Text	• Required: No • Read Only: No	Not applicable
Experiment Name	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable
Finish Date	Date	• Required: No • Read Only: Yes	Not applicable
Flow Cell ID	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable
Flow Cell Version	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable
Index 1 Read Cycles	Numeric	• Required: No • Read Only: Yes	Decimal Places Displayed: 0
Index 2 Read Cycles	Numeric	• Required: No • Read Only: Yes	Decimal Places Displayed: 0
Output Folder	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable
PR2 Bottle ID	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable
Read 1 Cycles	Numeric	• Required: No • Read Only: Yes	Decimal Places Displayed: 0
Read 2 Cycles	Numeric	• Required: No • Read Only: Yes	Decimal Places Displayed: 0
Reagent Cartridge ID	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable
Reagent Cartridge Part #	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable
Run ID	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Run Type	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable
Status	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable
Workflow	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable

Global UDFs/Custom Fields

There are several sample and measurement global UDFs/fields that are displayed on the Record Details screen of the MiSeq Run (MiSeq v3.0) step. These fields are autopopulated at the end of the sequencing run.

For more information, see *User Interaction* on page 2091 and *Sequencing Results Parsing* on page 2092.

Step File Placeholders

Placeholders for the following files are configured on the Record Details screen of the MiSeq Run (MiSeq v3.0) step:

- Illumina Run Report (autogenerated)
- Link to Run Folder (autogenerated)
- Run Parameters (autogenerated)
- Run Info (autogenerated)
- Lab Tracking Form (manually generated)
- Log File (autogenerated)

For details, see *Generated and Captured Files* on page 2092.

User Interaction

1. On the Queue screen, add the pool to the Ice Bucket, and then select View Ice Bucket.
2. On the Ice Bucket screen, select Begin Work.
3. On the Record Details screen, the fields in the table are read-only. After the run completes, the integration automatically performs the following actions:
    - Populates the fields.

- Attaches files to the Illumina Run Report, Link to Run Folder, Run Parameters, and Run Info placeholders.
- Populates the fields in the Sample Details table.

4. Select Next Steps.
   On the Assign Next Steps screen, the next step is already set to Mark protocol as complete.
5. Select Finish Step.

Sample Sheet Generation

Sample sheet generation occurs in the Denature & Dilute (MiSeq v3.0) step. This step places samples on the container loaded in the system.

The default configuration provides only the Validate Run Setup and Generate MiSeq SampleSheet automation. This automation uses the Template File Generator (`DriverFileGenerator.jar`) and a template file to generate a CSV format file for use with the MiSeq Control Software (MCS).

The sample sheet content is determined by the fields on the Record Details screen of the step in the Step Data table. The values entered into these fields are used to populate the sample sheet.

To customize the template used to create the sample sheet, you can insert additional columns.

There are additional details available:

- See *Illumina Instrument Sample Sheets (NGS v5.17 & later)* on page 2542, which provides a sample template that you can download and customize for the lab.
- For instructions on customizing the template, see *Creating Template Files* on page 2454.

Sequencing Results Parsing

The MiSeq Run (MiSeq v3.0) step records information for the flow cell lane and generates a report summarizing the results. In addition, run parameters, run info, and a link to the run folder are automatically captured.

Generated and Captured Files

The following table describes the run information files, reports, placeholders, and links that Clarity LIMS automatically generates or captures during a sequencing run.

Table 434 Run Information Generated or Captured by MiSeq Run (MiSeq v3.0) Step

Item	Description
Run Info Run Parameters	These XML files are automatically captured by Clarity LIMS from the run folder of the system. They include the key run parameters that are parsed out into step UDFs/custom fields in Clarity LIMS.

Item	Description
Link to Run Folder	This link is the path to the network run folder where the data that was captured from the system during the run is stored. The link is automatically generated by Clarity LIMS.
Illumina Run Report	This report provides key information about the run and the samples on the flow cell. The report it automatically generated by Clarity LIMS. Information includes the flow cell ID, run directory location, and primary analysis metrics for the sequencing run. Information is summarized per flow cell lane for the entire run and individual reads for paired-end runs. These metrics are compared against the per lane averages of the sequencing run. The per lane averages are calculated using metrics from the last five sequencing runs. Any values outside of one standard deviation are highlighted.
Lab Tracking Form placeholder	This placeholder in Clarity LIMS allows you to attach a lab-specific tracking form to the step manually.

Metadata

The following list shows metadata that Clarity LIMS automatically captures from the Illumina sequencing software as part of a sequencing run. This information is gathered from various run result files and events.

- Chemistry
- Experiment Name (entered in software)
- Finish Date (run completion date)
  - If the End Run event contains a date in the format YYYY-MM-DD, Finish Date is set to the date in the event file.
  - If the End Run event does not contain a date or the date is in the wrong format, Finish Date is set to the date when the event file is processed.
- Flow Cell ID
- Flow Cell Version
- Index 1 Read Cycles (intended Index cycles)
- Index 2 Read Cycles (intended Index cycles)
- Output Folder (run folder root)
- PR2 Bottle ID
- Reagent Cartridge ID

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- Reagent Cartridge Part #
- Read 1 Cycles
- Read 2 Cycles
- Run ID (the unique run ID)
- Run Type
- Status (current status of the sequencing run on the instrument)
- Workflow

Primary Analysis Metrics

The following table lists the Real-Time Analysis (RTA) primary analysis metrics Clarity LIMS automatically captures and records per read, for samples in each flow cell lane. These metrics are captured upon run completion and are stored as UDFs/custom fields in the Sample Details table on the Record Details screen.

To see both per read and per lane metrics, expand the output.

Table 435 RTA Primary Analysis Metrics Captured by MiSeq Run (MiSeq v3.0) Step

Per Read LIMS Field Name *(Stored on derived sample/analyte input to the step)*	Per Lane LIMS Field Name *(Stored in measurement placeholders in the Sample Details table on the Record Details screen)*
% Aligned R1	% Aligned R1
% Aligned R2	% Aligned R2
% Bases >=Q30 R1	% Bases >=Q30 R1
% Bases >=Q30 R2	% Bases >=Q30 R2
% Error Rate R1	% Error Rate R1
% Error Rate R2	% Error Rate R2
Not applicable	% Phasing R1
Not applicable	% Phasing R2
Not applicable	% Prephasing R1
Not applicable	% Prephasing R2
Not applicable	%PF R1
Not applicable	%PF R2
Not applicable	Cluster Density (K/mm^2) R1
Not applicable	Cluster Density (K/mm^2) R2

Per Read LIMS Field Name *(Stored on derived sample/analyte input to the step)*	Per Lane LIMS Field Name *(Stored in measurement placeholders in the Sample Details table on the Record Details screen)*
Intensity Cycle 1 R1	Intensity Cycle 1 R1
Intensity Cycle 1 R2	Intensity Cycle 1 R2
Not applicable	Reads PF (M) R1
Not applicable	Reads PF (M) R2
Yield PF (Gb) R1	Yield PF (Gb) R1
Yield PF (Gb) R2	Yield PF (Gb) R2

How It Works

1. The sequencing service runs on the Clarity LIMS server. The service detects event files that instrument RTA produces as the run progresses. The event files let the service know where to find the run data.
2. As the run data are written out and the End Run event is detected, the following events occur:
   - The data are matched to the step based on the reagent cartridge ID that was entered or scanned on the Denature and Dilute (MiSeq v3.0) step.
   - Read-only field values on the Record Details screen are populated accordingly.
3. When the service has finished processing the end run event and updating the fields in Clarity LIMS, the sequencing service generates the report and attaches it to the step.

User Interaction

1. Manually start the step, and then leave the step in progress until the sequencing service has finished processing the run and the run report is generated and attached.
2. After the run has completed, verify the following items:
   - The read-only fields on the Record Details screen are populated.
   - The Illumina Run Report is generated and attached.
   - The Link to Run Folder is generated and attached.
   - The Run Parameters and Run Info files are attached.

   Leave the step as in progress until the Illumina Run Report has been attached to the step in Clarity LIMS.
3. [Optional] In the Files section, upload the Lab Tracking Form.
4. Select Next Steps.

On the Assign Next Steps screen, the next step is already set to Mark protocol as complete. The Log File is attached when the next step for samples has been assigned by the Next Step - Advance automation.

5. Select Finish Step.

Components Installed

This integration requires components installed with the Preconfigured Workflow Package (PCWP).

The IlluminaMiSeq Integration Package v8.0 RPM installs the scripts and files listed in the following table.

Table 436 Illumina MiSeq Integration Package v8.0 Scripts and Files Installed

Files Installed	Location & Description
`configure_extensions_miseq_sequencingservice-v8.sh`	/opt/gls/clarity/config/ Script that installs the service properties in the database.
`log4j.xml`	/opt/gls/clarity/extensions/Illumina_MiSeq/v7/SequencingService/conf File containing the settings for logging the sequencing jar.
`miseq-sequencing-v8.jar`	/opt/gls/clarity/extensions/Illumina_MiSeq/v8/SequencingService Jar file containing API-based Clarity LIMS extensions used for capturing run results and report generation.
InterOp libraries	/opt/gls/clarity/extensions/Illumina_MiSeq/v8/lib Illumina shared library for parsing InterOp data files.
`MiSeqSamplesheetv3.csv` (Installed by the PCWP or IPP)	/opt/gls/clarity/extensions/conf/driverfiletemplates Template file used for file generation.

Database Properties Installed

The database properties installed in the Illumina MiSeq Integration v8.0 are described in the following section.

The integration also installs several additional database properties, each with the 99 suffix appended to their name, that are not listed in the table. These properties are intended for use by the Clarity LIMS support team in automated validation tests.

Sequencing runs are matched using the reagent cartridge ID and the base name of the sequencing step, MiSeq Run (MiSeq v3.0).

Do not change the base name. The sequencing service that captures sequencing run results expects this name. The base name is stored in the `sequenceProcessBaseName` database property. If the name is changed without the database property being updated, the reagent cartridge ID <-> sequencing step base name matching system fails.

If you need to rename the step, for example to add a version number, modify the text after the base name portion. This part of the name is not used in the matching system. For example, change MiSeq Run (MiSeq v2.0) to MiSeq Run (MiSeq v3.0).

Table 437 Database Properties Installed with the Illumina MiSeq Integration Package

Property	Description	Default Value
`miseq.v8.seqservice.sequenceProcessBaseName`	Sequencing process type/master step base display name. Partial matching is used to look up the process type/master step.	MiSeq Run (MiSeq v3.0)
`miseq.v8.seqservice.eventFileDirectory.1`	A network location monitored for event files, eg, `/mnt/illumina/gls_events/`	/mnt/gls_events
`miseq.v8.seqservice.netPathPrefixSearch.1`	The network directory prefix contained in the event file.	\\nas\network\run_data
`miseq.v8.seqservice.netPathPrefixReplace.1`	The mapped network directory mount name on the server used to access the run data directory, eg, `/mnt/network/data`	/mnt/run_data

Property	Description	Default Value
`miseq.v8.seqservice.eventFileDirectorySuffixes`	A list of eventFileDirectory path entries to monitor for event files. The value is one or more comma-separated integers.	99 Configured on install to point to 1
`miseq.v8.seqservice.netPathPrefixSearchReplaceSuffixes`	A list of netPathPrefix search and replace entries for transforming Windows to Linux network paths. The value is one or more comma-separated integers.	99 Configured on install to point to 1
`miseq.v8.seqservice.runReportViewsVersion`	The current version of the Run Report views in the database. The value 0 represents the state before the views are created. This property is automatically updated by the run report.	0

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Property	Description	Default Value
`miseq.v8.seqservice.ignoreUnmatchedContainerIds`	A flag indicating if event files that cannot be matched to reagent cartridges in Clarity LIMS should be archived after a certain period (true) or continually reprocessed (false).	False To prevent the gls_events file directory from becoming cluttered, it is recommended that the value of this property is set to true
`miseq.v8.seqservice.ignoreUnmatchedContainerIdsWaitDays`	The number of days between when the event is created and the event file is archived.	14
`miseq.v8.seqservice.synchronizationPeriod`	Invocation period in seconds.	60

You can configure support for multiple, identical seqservice.netPathPrefixSearch property values. For more information, see *Configure Multiple Identical netPathPrefixSearch Values* on page 2596

Consumables Installed

Reagent Categories and Kits

Reagent categories or label groups are installed with the Preconfigured Workflow Package (PCWP) workflow configuration slices.

The MiSeq Reagent Kit is included in the Illumina MiSeq Integration.

Control Types

The PhiX v3 control type is included in the Illumina MiSeq Integration.

Container Types

The MiSeq Reagent Cartridge container type is included in the Illumina MiSeq Integration.

All one-dimensional container types with both numeric rows and numeric columns are supported.

Instrument Integration

To make sure that the Illumina instrument warranty remains valid, the instrument integration must be performed and maintained by the Clarity LIMS Support team. To perform this integration, the Support team requires remote access to the system while it is idle.

The following steps are performed by the Clarity LIMS Support team when configuring the sequencing for use with the Illumina MiSeq Integration.

1. Create a directory on the local computer to hold the batch files. These batch files write event files to the network attached storage (NAS) shares.
2. Create a directory on the NAS to hold the event files.
3. Modify Illumina software configuration files to call the batch files that create the event files.
4. Update sequencing service default database properties to match the specifics of the installation.

Rules and Constraints

The Illumina MiSeq Integration operates with the following constraints:

- The reagent cartridge ID must be unique. There should not be multiple reagent cartridge containers in the system with the same name.
- The reagent cartridge ID must be scanned as the reagent cartridge Container Name on the Denature & Dilute (MiSeq v3.0) step.

MiSeq Integration v8.0 Validation and Troubleshooting

The following sections explain how to validate the installation of the Illumina MiSeq Integration Package v8.0.

The validation involves the following processes:

- Running samples through the Library Prep Validation workflow. The workflow contains a single-step protocol that models the library prep required to produce normalized libraries. At the end of the step, the normalized libraries automatically advance to the MiSeq Sequencing v3.0 workflow.
- Running normalized libraries through the MiSeq Sequencing v3.0 workflow. This process validates the following information:
  - Automated generation of a sample sheet for use with the MiSeq Control Software (MCS) and Local Run Manager (LRM).
  - Automated tracking of the MiSeq sequencing run and parsing of run statistics into Clarity LIMS, including the following information:
    - Run status and metrics of sequencing run
    - Sequencing run parameters

  - Real-Time Analysis (RTA) v1 run directory location and other run-specific information

The version of Library Prep Validation workflow depends on whether the workflow is delivered by PreConfigured Workflows Package (PCWP) or Illumina Preset Protocols (IPP).

- Library Prep Validation v1.1.2 (PCWP)
- Library Prep Validation v2.0.2 (IPP)

Activate Workflow, Create Project, Add and Assign Samples

The following steps prepare Clarity LIMS to run samples through the Library Prep Validation and MiSeq Sequencing v3.0 workflows.

1. In the Clarity LIMS Configuration area, activate the Library Prep Validation and MiSeq Sequencing v3.0 workflows.
   - In Clarity LIMS v4, select the workflow and select Activate.
   - In Clarity LIMS v5, select the workflow and change the Workflow Status toggle to Activate.
2. On the Projects & Samples screen, create a project, and then add samples to the project.
3. Assign the samples to the Library Prep Validation workflow.

Library Prep Validation Protocol

This single-step protocol models the library prep required to produce normalized libraries that are ready for the MiSeq Sequencing v3.1 workflow.

Run Library Prep Validation Step

1. In Lab View, locate the Library Prep Validation protocol. Samples are queued for the Library Prep Validation step. Select the step to proceed to the Queue screen.
2. On the Queue screen, add the samples to the Ice Bucket, and then select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Placement screen, move the samples as follows.
   a. In the container field in the Placed Samples area, scan or enter the barcode of the destination container.
   b. Select samples from the Samples to be Placed area and drag them to the container wells on the right. Repeat until all samples are placed.
   c. Select Add Labels.
5. On the Add Labels screen, assign the labels as follows.
   a. To assign labels, select a label and drag it to a container well. Repeat as needed.
   b. Select Record Details.
6. On the Record Details screen, set the samples to MiSeq as follows.
   a. Select all the samples in the Sample Details table.

b. In the Sequencing Instrument drop-down list, select MiSeq.
c. Select Apply to set this value for all selected samples.
d. Select Next Steps.
On the Assign Next Steps screen, the next step is automatically set to Remove from Workflow. This setting is required by the Routing script automation.

7. Select Finish Step.
The Routing script automation is triggered and samples are automatically routed to the first step in the MiSeq Sequencing v3.1 protocol. This is the Library Pooling (MiSeq v3.1) step.

MiSeq Sequencing v3.1 Protocol

Run Library Pooling (MiSeq v3.1)

1. In Lab View, locate the MiSeq Sequencing v3.1 protocol. Samples are queued for the Library Pooling (MiSeq v3.1) step. Select the step to proceed to the Queue screen.
2. On the Queue screen, do as follows.
   a. Add the samples to the Ice Bucket.
   b. In the Add Control Samples panel, add the PhiX v3 control sample to the Ice Bucket.
   c. Select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
   The Validate Maximum Number of Samples automation script runs. The maximum number of samples allowed is 1536.
4. On the Pool Samples screen, create a pool of samples as follows.
   a. Create a pool of samples by dragging samples into the Pool Creator.
   b. Name the pool. You can also accept the default name Pool #1.
   c. Select Place Samples.
5. On the Placement screen, move the pool to the container as follows.
   a. Select the pool from the Samples to be Placed area and drag it to the container.
   b. Select Record Details.
6. On the Record Details screen, select Next Steps.
   On the Assign Next Steps screen, next step is automatically set to Denature and Dilute (MiSeq v3.1).
7. Select Finish Step.

Run Denature and Dilute (MiSeq v3.1)

1. In Lab View, locate the MiSeq Sequencing v3.1 protocol. The pooled samples are queued for the Denature and Dilute (MiSeq v3.1) step. Select the step to proceed to the Queue screen.
2. On the Queue screen, add the pool to the Ice Bucket. Select View Ice Bucket.

3. On the Ice Bucket screen, select Begin Work.
4. On the Placement screen, move the pool to the reagent cartridge as follows.
   a. Scan the MiSeq reagent cartridge barcode into the MiSeq Reagent Cartridge field.
   b. Place the pool of samples into the reagent cartridge.
   c. Select Record Details.
5. On the Record Details screen, create the sample sheet as follows.
   a. In Reagent Lot Tracking, select the reagent lot used in the step. You may need to add/activate the lot on the Reagents and Controls screen.
   b. In the Step Details table, populate the fields as appropriate. Workflow, Experiment Name, and Read 1 Cycles are required fields. For more information on presets, see *Using Step Details Presets* on page 2103.
   c. In the Sample Details table, enter the Final Loading Concentration. You may select preset 225 (for PCR-free workflows) or preset 400 (for Nano workflows). You can also enter a different value.
   d. Select Validate Run Setup and Generate MiSeq SampleSheet. Clarity LIMS generates the sample sheet and attaches it and a log file to placeholders in the Files area of the Record Details screen.
   e. Download the files and validate the format and content.
6. Select Next Steps.
   On the Assign Next Steps screen, make sure samples are automatically assigned to the MiSeq Run (MiSeq v3.1) step.
7. Select Finish Step.

Using Step Details Presets

Most of the fields in Step Details are analysis settings used by Local Run Manager and are for advanced users only. The following fields are exceptions:

- Workflow
- Experiment Name
- Description
- Read 1 Cycles
- Read 2 Cycles
- Custom Primers

To use the default analysis settings, select the analysis module from the Preset drop-down list. The default values populate the respective fields and indicate the following actions are needed:

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- Required—The field is required for the specific analysis module selected. Replace the word Required with a proper value. For example, the Genome Folder field should contain the directory to the genome folder.
- None—The field is not applicable for the specific analysis module selected. Leave the field as is.

For a list of UDFs that are applicable for the analysis module selected, refer to the Applicable analysis fields for the selected Workflow field in Step Details.

Run MiSeq Run (MiSeq v3.1)

1. In Lab View, locate the MiSeq Sequencing v3.1 protocol. The pool of samples is queued for the MiSeq Run (MiSeq v3.1) step. Select the step to proceed to the Queue screen.
2. On the Queue screen, add the pool to the Ice Bucket. Select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Record Details screen, the fields are read-only. When the run completes, the integration automatically:
   - Populates the fields.
   - Attaches files to the Illumina Run Report, Link to Run Folder, Run Parameters, and Run Info placeholders.
   - Populates the fields in the Sample Details table. See *MiSeq Integration v8.0 Configuration and User Interaction* on page 2066 for details.

   The Log File is attached after the next step for samples is assigned by the Next Step - Advance automation. Do not continue to step 5 and complete the step until the Illumina Run Report has been attached.
5. Select Next Steps.
   On the Assign Next Steps screen, the next step is automatically set to Mark protocol as complete.
6. Select Finish Step.

Validating Creation of Event Files

This validation checks the following information:

- The destination path is correctly configured.
- The instrument computer can access and write to the destination path.
- There are no syntax errors in the Clarity LIMS batch file.

Use the following steps to confirm that event files are created by the batch file in the destination path. The steps assume that the default configuration has been successfully imported.

1. In `C:\Illumina\gls`, double-click the batch file `gls_event_mcs_rta_lrm.bat`.
2. Confirm that an empty event file appears in the configured DESTINATION_PATH.

Manual execution of the batch file produces an output resembling the following example:

```
Filename:
```


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```
event-EndRun-07295667.txt
Contents:
cycleNumber =
runFolder =
netFolder =
readType =
eventType = EndRun
softwareType = MCS
finishDate = 2021-01-25
```

Troubleshooting

If an automation trigger does not appear to run the corresponding scripts, see the following topics:

- *Troubleshooting Automated Worker* in the Clarity LIMS Core Functionality documentation.
- *Troubleshooting Automation* in the Clarity LIMS API & Database documentation.

If an error occurs that does not provide direction on how to proceed, confirm the version of the installed MiSeq Integration Package by running the `rpm -qa | grep -i miseq` command from the server console.

If the error is related to data parsing, retrieving run results data, or report values not appearing as expected, review the `MiseqIntegrator.log` file. It is located at `/opt/gls/clarity/extensions/Illumina_MiSeq/v8/SequencingService`.

Additional troubleshooting information for this integration is provided on the *Illumina Instrument Integrations FAQ* on page 2598 page.

If you are unable to resolve the issue, contact the Clarity LIMS support team. Make sure you supply the relevant information from the troubleshooting that was already performed.

MiSeq Integration v8.0 Release Notes

The following release notes detail the key changes to software components for the Clarity LIMS MiSeq Integration package v8.0.

Compatibility

- Clarity LIMS v4 (4.2 or later)
- Clarity LIMS v5 (v5.1 or later)
- NGS Extensions Package v5.20 or later
- MiSeq Control Software (MCS) v4.0
- Local Run Manager v3.0
- Illumina Preset Protocols (IPP) v2.1
- Preconfigured Workflow Package (PCWP) v2.1

New Features

The main goal of this release of the Clarity LIMS MiSeq integration along with the latest version of the default MiSeq Workflow is to support the integration between Clarity LIMS, Miseq instrument, and Windows 10.

The following Local Run Manager Modules are supported with this release:

- GenerateFastQ
- Library QC
- Resequencing
- DNA Enrichment
- DNA Amplicon

Defects Fixed

- The correct network folder path displays in the `EndRun.txt` event file.
- The script `configure_extensions_miseq_sequencingservice-v8.sh` can now be executed as glsjboss user.
- The `RunReport.pdf` now contains the current output folder path

Known Issues

- None

MiSeq Integration v7.1 Configuration and User Interaction

The Illumina MiSeq Integration Package v7.1 supports the integration of Clarity LIMS to Illumina MiSeq Sequencing Systems.

The integration allows for automated tracking of an Illumina sequencing run in Clarity LIMS. This capability includes tracking sequencing run status, generating run report, and capturing and parsing run statistics. In addition, this integration provides automated generation of a sample sheet file for use with the MiSeq Control Software (MCS).

Overview

The Illumina MiSeq Integration Package v7.1 includes the following features:

- Preconfigured MiSeq Sequencing v2.0 workflow that maps to lab protocol steps and sequencing runs.
- Automated generation of a sample sheet for use with the MiSeq Control Software (MCS).
- Automated tracking of the MiSeq sequencing run and parsing of run statistics into Clarity LIMS, including:

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  - Run status and metrics of sequencing run
  - Sequencing run parameters
  - Real-Time Analysis (RTA) v1 run directory location and other run-specific information

This integration also includes a preconfigured Library Prep Validation workflow that is used for validation purposes only. The workflow contains a single-step protocol that models the library prep required to produce normalized libraries that are ready for the MiSeq Sequencing v2.0 workflow. For details, see *MiSeq Integration v7.0 Validation and Troubleshooting* on page 2158.

Depending on the Clarity LIMS version, install the workflows as follows.

- For Clarity LIMS v4 (4.2 and later), install the following workflows via Preconfigured Workflows Package (PCWP) v2.0.1:
  - MiSeq Sequencing v2.0 workflow
  - Library Prep Validation v1.1 workflow
- For Clarity LIMS v5 (5.1 and later), install the following workflows via Illumina Preset Protocols (IPP) v2.0:
  - MiSeq Sequencing v2.0 workflow
  - Library Prep Validation v2.0 workflow
- For Clarity LIMS v5.0.x, contact Illumina Technical Support to upgrade your integration.

The following sections describe the MiSeq Sequencing v2.0 workflow and the integration between Clarity LIMS and the Illumina MiSeq system.

For instructions on validating and troubleshooting the Illumina MiSeq Integration Package, see *MiSeq Integration v7.0 Validation and Troubleshooting* on page 2158.

Prerequisites and Assumptions

Before samples are assigned to the workflow, make sure that the following prerequisites are completed.

- Samples have been run through QC and library prep.
- Samples have been normalized, and the value is captured in a field called Normalized Molarity (nM).
- Reagent labels are attached to samples.
- Samples have been accessioned into Clarity LIMS. For more information on sample accessioning, see *Sample Accessioning* in the Getting Started section of the Clarity LIMS Core Functionality documentation.

Samples can be assigned to the MiSeq Sequencing v2.0 workflow automatically using a routing script or manually from the Projects & Samples dashboard. See *Assign and Process Samples* in the Getting Started section of the Clarity LIMS Core Functionality documentation.

Workflows, Protocols, and Steps

The Illumina MiSeq Integration includes the MiSeq Sequencing v2.0 workflow and the MiSeq Sequencing v2.0 protocol.

The protocol includes the following steps and automations.

Table 438 MiSeq Sequencing v2.0 Protocol Steps and Automations

Step	Automation
1. Library Pooling (MiSeq v2.0)	• Set Next Step - Advance
2. Denature & Dilute (MiSeq v2.0)	• Generate MiSeq SampleSheet • Set Next Step - Advance
3. MiSeq Run (MiSeq v2.0)	• Set Next Step - Advance

Terminology

This integration supports Clarity LIMS v4.2 and later.

As of Clarity LIMS v5, the term user-defined field (UDF) has been replaced with custom field in the user interface. However, the API resource is still called UDF.

There are two types of custom fields:

- Master step fields—Configured on master steps. Master step fields only apply to the following:
  - The master step on which the fields are configured.
  - The steps derived from those master steps.
- Global fields—Configured on entities (eg, submitted sample, derived sample, measurement, etc.). Global fields apply to the entire Clarity LIMS system.

Step 1: Library Pooling (MiSeq v2.0)

In Clarity LIMS v5, the Library Pooling (MiSeq v2.0) step is derived from the Library Pooling v2.0 master step.

In this step, the lab scientist manually places libraries into pools in the Clarity LIMS Placement screen.

Set Next Step - Advance Automation

This automation advances samples to the next step in the protocol. The automation is automatically triggered on exit of the Record Details screen.

Default command line:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-t false \
```


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```
-h false \
-exp 'nextStep = ::ADVANCE::' \
-log {compoundOutputFileLuid0}"
```

Step UDFs/Custom Fields

The Comment field is configured on the Library Pooling (MiSeq v2.0) step. The comment field displays on the Record Details screen at run time.

Table 439 Library Pooling (MiSeq v2.0) Step UDF/Custom Field Configuration

Field Name	Field Type	Field Constraints/Options
Comment	Multiline Text	• Required: No • Read Only: No

Global UDFs/Custom Fields

The following table lists the global UDFs/fields that are displayed on the Queue and Ice Bucket screens of the Library Pooling (MiSeq v2.0) step. Most fields display in expanded view only.

Table 440 Global UDF/Custom Field Configuration for Library Pooling (MiSeq v2.0)

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Configured on Sample in Clarity LIMS v4 and Submitted Sample in Clarity LIMS v5:			

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Application	Single-line Text (v4) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: Yes	• TruSeq mRNA sequencing • TruSeq DNA sequencing (large genome de novo) • TruSeq DNA sequencing (large genome reseq) • TruSeq DNA sequencing (small genome de novo) • TruSeq DNA sequencing (small genome reseq) • Nextera DNA sequencing • TruSeq Custom Amplicon sequencing • ChIP-sequencing • Exome sequencing • Mate pair sequencing • Small RNA sequencing
Pooling	Single-line Text (v4) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: Yes	• Yes • No
Read Length	Single-line Text (v4) Text (v5)	• Required: No • Read Only: No	Not applicable
Sequencing Coverage	Single-line Text (v4) Text (v5)	• Required: No • Read Only: No	Not applicable

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Sequencing Method	Single-line Text (v4) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: Yes	Not applicable
Configured on Analyte in Clarity LIMS v4 and Derived Sample in Clarity LIMS v5:			
Normalized Molarity (nM)	Numeric	• Required: No • Read Only: No	Decimal Places Displayed: 2

User Interaction

1. On the Queue screen, do as follows.
   a. Add the samples to the Ice Bucket.
   b. [Optional] In the Add Control Samples panel, add the PhiX v3 control sample to the Ice Bucket.
   c. Select View Ice Bucket.
2. On the Ice Bucket screen, select Begin Work.
3. On the Pool Samples screen, do as follows.
   a. Create a pool of samples by dragging samples into the Pool Creator.
   b. Name the pool or accept the default name (Pool #1).
   c. Select Place Samples.
4. On the Placement screen, do as follows.
   a. Select the pool in the Samples to be Placed area and drag it to the container.
   b. Select Record Details.
5. On the Record Details screen, select Next Steps.
   On the Assign Next Steps screen, next step is already set to Denature and Dilute (MiSeq v2.0).
6. Select Finish Step.

Step 2: Denature and Dilute (MiSeq v2.0)

In this step, pooled libraries are denatured and diluted, and then placed into the reagent cartridge loaded into the MiSeq instrument.

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Generate MiSeq SampleSheet Automation

This automation generates the sample sheet and attaches it to the Denature and Dilute step. Select a button on the Record Details screen to trigger this automation. For details, see *Sample Sheet Generation* on page 2124.

Default command line:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p {password} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/MiSeqSamplesheetv2.csv \
-o {compoundOutputFileLuid1}.csv \
-q true \
-destLIMSID {compoundOutputFileLuid1} \
-l {compoundOutputFileLuid2}"
```

Set Next Step - Advance Automation

This automation advances samples to the next step in the protocol. The automation is automatically triggered by exiting the Record Details screen.

In Clarity LIMS v4, the automation also invokes the setUDF script, which sets the value of the Progress UDF. Clarity LIMS updates this UDF value as samples progress through the workflow steps. The Progress UDF does not display in the Clarity LIMS user interface but does display in the LabLink interface. This feature allows collaborators to see the status of samples.

Default command line (Clarity LIMS v4):

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -u {username} -p {password} \
script:setUDF \
-i {processURI:v2} \
-f 'Progress' \
-t '//input/@uri->//sample/@uri' \
-v 'Library ready for sequencing' \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -u {username} -p {password} \
script:evaluateDynamicExpression \
-i {stepURI:v2} \
-t false \
-h false \
-exp 'nextStep = ::ADVANCE::' \
-log {compoundOutputFileLuid3}"
```

Default command line (Clarity LIMS v5):

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-t false \
-h false \
-exp 'nextStep = ::ADVANCE::' \
-log {compoundOutputFileLuid3}"
```

Step UDFs/Custom Fields

The fields configured on the Denature & Dilute (MiSeq v2.0) step display on the Record Details screen in the Step Data table. These fields are manually populated at run time (see *User Interaction* on page 2121).

The values are then used to generate the sample sheet. For details on the sample generation functionality, see *Sample Sheet Generation* on page 2124.

Table 441 Denature & Dilute (MiSeq v2.0) Step UDF/Custom Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Adapter	Single-line Text (v4) Text (v5)	• Required: No • Read Only: No	Not applicable
Assay	Single-line Text (v4) Text (v5)	• Required: No • Read Only: No	Not applicable
Comment	Multiline Text	• Required: No • Read Only: No	Not applicable
Control CAT Manifest	Single-line Text (v4) Text (v5)	• Required: No • Read Only: No	Not applicable
Custom CAT Manifest	Single-line Text (v4) Text (v5)	• Required: No • Read Only: No	Not applicable

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Custom Primers	Single-line Text (v4) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: No	• None (Default) • Read 1 • Index • Read 2 • Read 1, Index • Read 1, Index, Read 2 • Read 1, Read 2 • Index, Read 2
Description	Single-line Text (v4) Text (v5)	• Required: No • Read Only: No	Not applicable
Experiment Name	Single-line Text (v4) Text (v5)	• Required: Yes • Read Only: No	Not applicable
FilterPCRDuplicates	Checkbox (v4) Toggle Switch (v5)	• Required: No • Read Only: No	• Default: None Set
GenomeFolder	Single-line Text (v4) Text (v5)	• Required: No • Read Only: No	Not applicable
MinQScore	Numeric	• Required: No • Read Only: No	• Range From: 0 to 100 • Decimal Places Displayed: 2
OnlyGenerateFASTQ	Checkbox (v4) Toggle Switch (v5)	• Required: No • Read Only: No	• Default: None Set
Read 1 Cycles	Numeric (v4) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 251 (Default) • 151 • 101 • 76 • 51 • Range From: 0 to 1000 • Decimal Places Displayed: 0

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Read 2 Cycles	Numeric (v4) Numeric Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: Yes	• 251 • 151 • 101 • 76 • 51 • Range From: 0 to 1000 • Decimal Places Displayed: 0
SiteReports *Hidden field, does not display on Record Details screen*	Single-line Text (v4) Text (v5)	• Required: No • Read Only: No	Not applicable
StitchReads *Only for GenerateFASTQ, Amplicon DS and TruSeqAmplicon workflows*	Checkbox (v4) Toggle Switch (v5)	• Required: No • Read Only: No	• Default: None Set
Use submitted sample details for Genome Folder location	Checkbox (v4) Toggle Switch (v5)	• Required: No • Read Only: No	• Default: None Set
VariantCaller	Single-line Text (v4) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: No	• GATK (Default) • Somatic • Starling • None
Workflow	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• Assembly • Custom Amplicon • Enrichment • GenerateFASTQ • LibraryQC • Metagenomics • PCR Amplicon • Resequencing

Groups of Defaults/Presets

The following groups of defaults/presets are configured for Denature & Dilute (MiSeq v2.0) step UDFs/master step custom fields:

Resequencing

Field	Configuration
Custom Primers	None
FilterPCRDuplicates	Yes
GenomeFolder	Required
OnlyGenerateFastQ	No
Read 1 Cycles	251
StitchReads	None Set
Use submitted sample details for Genome Folder location	No
Workflow	Resequencing

Custom Amplicon

Field	Configuration
Custom CAT Manifest	Required
Custom Primers	None
FilterPCRDuplicates	Yes
GenomeFolder	Required
OnlyGenerateFastQ	No
Read 1 Cycles	251
StitchReads	None Set
Use submitted sample details for Genome Folder location	No
Workflow	Custom Amplicon

PCR Amplicon

Field	Configuration
Custom CAT Manifest	Required
Custom Primers	None
FilterPCRDuplicates	Yes
GenomeFolder	Required
OnlyGenerateFastQ	No
Read 1 Cycles	251
StitchReads	None Set
Use submitted sample details for Genome Folder location	No
Workflow	PCR Amplicon

Enrichment

Field	Configuration
Custom CAT Manifest	Required
Custom Primers	None
FilterPCRDuplicates	Yes
GenomeFolder	Required
OnlyGenerateFastQ	No
Read 1 Cycles	251
StitchReads	None Set
Use submitted sample details for Genome Folder location	No
Workflow	Enrichment

Library QC

Field	Configuration
Custom Primers	None
FilterPCRDuplicates	Yes
GenomeFolder	Required

Field	Configuration
OnlyGenerateFastQ	No
Read 1 Cycles	251
StitchReads	None Set
Use submitted sample details for Genome Folder location	No
Workflow	LibraryQC

Metagenomics

Field	Configuration
Custom Primers	None
FilterPCRDuplicates	Yes
OnlyGenerateFastQ	No
Read 1 Cycles	251
StitchReads	None Set
Use submitted sample details for Genome Folder location	No
Workflow	Metagenomics

GenerateFastQ

Field	Configuration
Custom Primers	None
FilterPCRDuplicates	Yes
OnlyGenerateFastQ	Yes
Read 1 Cycles	251
StitchReads	None Set
Use submitted sample details for Genome Folder location	No
Workflow	GenerateFastQ

Assembly

Field	Configuration
Custom Primers	None
FilterPCRDuplicates	Yes
OnlyGenerateFastQ	No
Read 1 Cycles	251
StitchReads	None Set
Use submitted sample details for Genome Folder location	No
Workflow	Assembly

Global UDFs/Custom Fields

The following table shows the global UDFs/fields that are configured to display on the Queue, Ice Bucket, and Record Details screens of the Denature & Dilute (MiSeq v2.0) step.

Table 442 Global UDF/Custom Field Configuration for Denature & Dilute (MiSeq v2.0) Step

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Configured on Sample in LIMS v4 and Submitted Sample in LIMS v5:			
Progress *(LIMS v4) Displays in LabLink Collaborations Interface only*	Single-line Text (v4) Text (v5)	• Required: No • Read Only: No • Custom Entries: Yes	Not applicable
Read Length *Displays on Queue & Ice Bucket screens*	Single-line Text (v4) Text (v5)	• Required: No • Read Only: No	Not applicable
Sequencing Method *Displays on Queue & Ice Bucket screens*	Single-line Text (v4) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: Yes	• Single Read • Paired End Read • Indexed Single Read • Indexed Paired End Read

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Configured on Analyte in LIMS v4 and Derived Sample in LIMS v5:			
Final Loading Concentration *Displays on Record Details screen*	Numeric (v4) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 225 • 400

Step File Placeholders

Placeholders for the following files are configured on the Record Details screen of the Denature & Dilute (MiSeq v2.0) step:

- Lab Tracking Form (manually generated)
- MiSeq SampleSheet (autogenerated)
- MiSeq SampleSheet Generation Log (autogenerated)
- Log File (autogenerated)

Table 443 Sample Sheet Files and Placeholders Generated by Denature & Dilute (MiSeq v2.0) Step

Item	Description
Lab Tracking Form placeholder	This placeholder in Clarity LIMS allows the lab scientist to attach a lab-specific tracking form to the step manually.
MiSeq SampleSheet	This CSV file is automatically generated by Clarity LIMS for use with the MiSeq system. The file can be opened as a text file or an Excel spreadsheet.

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Item	Description
MiSeq SampleSheet Generation Log	This log file is automatically generated by Clarity LIMS. The log file captures any errors that Clarity LIMS might encounter when generating the sample sheet.
Log File	This log file is automatically generated by Clarity LIMS. The log file captures the status of the EvaluateDynamicExpression script that is invoked by the Set Next Step - Advance automation.

User Interaction

1. On the Queue screen, add the pool to the Ice Bucket, and then select View Ice Bucket.
2. On the Ice Bucket screen, select Begin Work.
3. On the Placement screen, do as follows.
   a. Scan the barcode of the MiSeq reagent cartridge into the MiSeq Reagent Cartridge field.
   b. Place the pool of samples into the reagent cartridge.
   c. Select Record Details.
4. On the Record Details screen, do as follows.
   a. Under Reagent Lot Tracking, select the reagent lot used in the step.
   If the reagent lot is not listed, add/activate the lot on the Reagents and Controls screen.
   b. Populate the fields in the Step Details table, as needed. Workflow and Read 1 Cycles are required fields.
   c. In the Sample Details table, enter the Final Loading Concentration. Select from the following preset options or enter a different value.
      - 225 (for PCR-free workflows)
      - 400 (for Nano workflows)
   d. Select Generate MiSeq SampleSheet.
   Clarity LIMS generates the sample sheet and attaches it and a log file to placeholders in the Files area of the Record Details screen.
   e. Download the files and validate their format and content.

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5. Select Next Steps.
   On the Assign Next Steps screen, samples are already assigned to the MiSeq Run (MiSeq v2.0) step.
6. Select Finish Step.

See also *Sample Sheet Generation* on page 2124.

Step 3: MiSeq Run (MiSeq v2.0)

Set Next Step - Advance Automation

This automation advances samples to the next step in the protocol. The automation is automatically triggered by exiting the Record Details screen.

Default command line:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-t false \
-h false \
-exp 'nextStep = ::ADVANCE::' \
-log {compoundOutputFileLuid5}"
```

Step UDFs/Custom Fields

There are 18 fields configured on the MiSeq Run (MiSeq v2.0) step. These fields display on the Record Details screen at run time.

The read-only fields are automatically populated at the end of the run.

Table 444 MiSeq Run (MiSeq v2.0) Step UDF/Custom Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Chemistry	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable
Comment	Multiline Text	• Required: No • Read Only: No	Not applicable
Experiment Name	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable

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Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Finish Date	Date	• Required: No • Read Only: Yes	Not applicable
Flow Cell ID	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable
Flow Cell Version	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable
Index 1 Read Cycles	Numeric	• Required: No • Read Only: Yes	Decimal Places Displayed: 0
Index 2 Read Cycles	Numeric	• Required: No • Read Only: Yes	Decimal Places Displayed: 0
Output Folder	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable
PR2 Bottle ID	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable
Read 1 Cycles	Numeric	• Required: No • Read Only: Yes	Decimal Places Displayed: 0
Read 2 Cycles	Numeric	• Required: No • Read Only: Yes	Decimal Places Displayed: 0
Reagent Cartridge ID	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable
Reagent Cartridge Part #	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable
Run ID	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable
Run Type	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable
Status	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable
Workflow	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable

Global UDFs/Custom Fields

There are several sample and measurement global UDFs/fields that are displayed on the Record Details screen of the MiSeq Run (MiSeq v2.0) step. These fields are autopopulated at the end of the sequencing run.

For more information, see *User Interaction* on page 2124 and *Sequencing Results Parsing* on page 2125.

Step File Placeholders

Placeholders for the following files are configured on the Record Details screen of the MiSeq Run (MiSeq v2.0) step:

- Illumina Run Report (autogenerated)
- Link to Run Folder (autogenerated)
- Run Parameters (autogenerated)
- Run Info (autogenerated)
- Lab Tracking Form (manually generated)
- Log File (autogenerated)

For details, see *Generated and Captured Files* on page 2125.

User Interaction

1. On the Queue screen, add the pool to the Ice Bucket, and then select View Ice Bucket.
2. On the Ice Bucket screen, select Begin Work.
3. On the Record Details screen, the fields in the table are read-only. After the run completes, the integration automatically performs the following actions:
   - Populates the fields.
   - Attaches files to the Illumina Run Report, Link to Run Folder, Run Parameters, and Run Info placeholders.
   - Populates the fields in the Sample Details table.
4. Select Next Steps.
   On the Assign Next Steps screen, the next step is already set to Mark protocol as complete.
5. Select Finish Step.

Sample Sheet Generation

Sample sheet generation occurs in the Denature & Dilute (MiSeq v2.0) step. This step places samples on the container loaded in the system.

The default configuration provides only the Generate MiSeq SampleSheet automation. This automation uses the Template File Generator (`DriverFileGenerator.jar`) and a template file to generate a CSV format file for use with the MiSeq Control Software (MCS).

The sample sheet content is determined by the fields on the Record Details screen of the step in the Step Data table. The values entered into these fields are used to populate the sample sheet.

To customize the template used to create the sample sheet, you can insert additional columns.

There are additional details available:

- See *Illumina Instrument Sample Sheets (NGS v5.17 & later)* on page 2542, which provides a sample template that you can download and customize for the lab.
- For instructions on customizing the template, see *Creating Template Files* on page 2454.

Sequencing Results Parsing

The MiSeq Run (MiSeq v2.0) step records information for the flow cell lane and generates a report summarizing the results. In addition, run parameters, run info, and a link to the run folder are automatically captured.

Generated and Captured Files

The following table describes the run information files, reports, placeholders, and links that Clarity LIMS automatically generates or captures during a sequencing run.

Table 445 Run Information Generated or Captured by MiSeq Run (MiSeq v2.0) Step

Item	Description
Run Info Run Parameters	These XML files are automatically captured by Clarity LIMS from the run folder of the system. They include the key run parameters that are parsed out into step UDFs/custom fields in Clarity LIMS.
Link to Run Folder	This link is the path to the network run folder where the data that was captured from the system during the run is stored. The link is automatically generated by Clarity LIMS.
Illumina Run Report	This report provides key information about the run and the samples on the flow cell. The report it automatically generated by Clarity LIMS. Information includes the flow cell ID, run directory location, and primary analysis metrics for the sequencing run. Information is summarized per flow cell lane for the entire run and individual reads for paired-end runs. These metrics are compared against the per lane averages of the sequencing run. The per lane averages are calculated using metrics from the last five sequencing runs. Any values outside of one standard deviation are highlighted.

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Item	Description
Lab Tracking Form placeholder	This placeholder in Clarity LIMS allows you to attach a lab-specific tracking form to the step manually.

Metadata

The following list shows metadata that Clarity LIMS automatically captures from the Illumina sequencing software as part of a sequencing run. This information is gathered from various run result files and events.

- Chemistry
- Experiment Name (entered in software)
- Finish Date (run completion date)
  - If the End Run event contains a date in the format YYYY-MM-DD, Finish Date is set to the date in the event file.
  - If the End Run event does not contain a date or the date is in the wrong format, Finish Date is set to the date when the event file is processed.
- Flow Cell ID
- Flow Cell Version
- Index 1 Read Cycles (intended Index cycles)
- Index 2 Read Cycles (intended Index cycles)
- Output Folder (run folder root)
- PR2 Bottle ID
- Reagent Cartridge ID
- Reagent Cartridge Part #
- Read 1 Cycles
- Read 2 Cycles
- Run ID (the unique run ID)
- Run Type
- Status (current status of the sequencing run on the instrument)
- Workflow

Primary Analysis Metrics

The following table lists the Real-Time Analysis (RTA) primary analysis metrics Clarity LIMS automatically captures and records per read, for samples in each flow cell lane. These metrics are captured upon run completion and are stored as UDFs/custom fields in the Sample Details table on the

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Record Details screen.

To see both per read and per lane metrics, expand the output.

Table 446 RTA Primary Analysis Metrics Captured by MiSeq Run (MiSeq v2.0) Step

Per Read LIMS Field Name *(Stored on derived sample/analyte input to the step)*	Per Lane LIMS Field Name *(Stored in measurement placeholders in the Sample Details table on the Record Details screen)*
% Aligned R1	% Aligned R1
% Aligned R2	% Aligned R2
% Bases >=Q30 R1	% Bases >=Q30 R1
% Bases >=Q30 R2	% Bases >=Q30 R2
% Error Rate R1	% Error Rate R1
% Error Rate R2	% Error Rate R2
Not applicable	% Phasing R1
Not applicable	% Phasing R2
Not applicable	% Prephasing R1
Not applicable	% Prephasing R2
Not applicable	%PF R1
Not applicable	%PF R2
Not applicable	Cluster Density (K/mm^2) R1
Not applicable	Cluster Density (K/mm^2) R2
Intensity Cycle 1 R1	Intensity Cycle 1 R1
Intensity Cycle 1 R2	Intensity Cycle 1 R2
Not applicable	Reads PF (M) R1
Not applicable	Reads PF (M) R2
Yield PF (Gb) R1	Yield PF (Gb) R1
Yield PF (Gb) R2	Yield PF (Gb) R2

How It Works

1. The sequencing service runs on the Clarity LIMS server. The service detects event files that instrument RTA produces as the run progresses. The event files let the service know where to find the run data.
2. As the run data are written out and the End Run event is detected, the following events occur:

- The data are matched to the step based on the reagent cartridge ID that was entered or scanned on the Denature & Dilute (MiSeq v2.0) step.
- Read-only field values on the Record Details screen are populated accordingly.

3. When the service has finished processing the end run event and updating the fields in Clarity LIMS, the sequencing service generates the report and attaches it to the step.

User Interaction

1. Manually start the step, and then leave the step in progress until the sequencing service has finished processing the run and the run report is generated and attached.
2. After the run has completed, verify the following items:
   - The read-only fields on the Record Details screen are populated.
   - The Illumina Run Report is generated and attached.
   - The Link to Run Folder is generated and attached.
   - The Run Parameters and Run Info files are attached.

   Leave the step as in progress until the Illumina Run Report has been attached to the step in Clarity LIMS.
3. [Optional] In the Files section, upload the Lab Tracking Form.
4. Select Next Steps.
   On the Assign Next Steps screen, the next step is already set to Mark protocol as complete. The Log File is attached when the next step for samples has been assigned by the Next Step - Advance automation.
5. Select Finish Step.

Components Installed

This integration requires components installed with the Preconfigured Workflow Package (PCWP).

The IlluminaMiSeq Integration Package v7.1 RPM installs the scripts and files listed in the following table.

Table 447 Illumina MiSeq Integration Package v7.1 Scripts and Files Installed

Files Installed	Location & Description
`configure_extensions_miseq_sequencingservice-v7.sh`	/opt/gls/clarity/config/ Script that installs the service properties in the database.
`log4j.xml`	/opt/gls/clarity/extensions/Illumina_MiSeq/v7/SequencingService/conf File containing the settings for logging the sequencing jar.

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Files Installed	Location & Description
`miseq-sequencing-v7.jar`	/opt/gls/clarity/extensions/Illumina_MiSeq/v7/SequencingService Jar file containing API-based Clarity LIMS extensions used for capturing run results and report generation.
InterOp libraries	/opt/gls/clarity/extensions/Illumina_MiSeq/v7/lib Illumina shared library for parsing InterOp data files.
`MiSeqSamplesheetv2.csv` (Installed by the PCWP or IPP)	/opt/gls/clarity/extensions/conf/driverfiletemplates Template file used for file generation.

Database Properties Installed

The Illumina MiSeq Integration v7.1 also installs several additional database properties, each with the 99 suffix appended to their name. These properties are intended for use by the Clarity LIMS support team in automated validation tests and are not listed in the table.

Sequencing runs are matched using the reagent cartridge ID and the base name of the sequencing step, MiSeq Run (MiSeq v2.0).

Do not change the base name. The sequencing service that captures sequencing run results expects this name. The base name is stored in the `sequenceProcessBaseName` database property. If the name is changed without the database property being updated, the reagent cartridge ID <-> sequencing step base name matching system fails.

If you need to rename the step, for example to add a version number, modify the text after the base name portion. This part of the name is not used in the matching system. For example, change MiSeq Run (MiSeq v2.0) to MiSeq Run (MiSeq v2.0) v2.

Table 448 Database Properties Installed with the Illumina MiSeq Integration Package

Property	Description	Default Value
`miseq.v7.seqservice.sequenceProcessBaseName`	Sequencing process type/master step base display name. Partial matching is used to look up the process type/master step.	MiSeq Run (MiSeq v2.0)

Property	Description	Default Value
`miseq.v7.seqservice.eventFileDirectory.1`	A network location monitored for event files, eg, `/mnt/illumina/gls_events/`	/mnt/gls_events
`miseq.v7.seqservice.netPathPrefixSearch.1`	The network directory prefix contained in the event file.	\\nas\network\run_data
`miseq.v7.seqservice.netPathPrefixReplace.1`	The mapped network directory mount name on the server used to access the run data directory, eg, `/mnt/network/data`	/mnt/run_data
`miseq.v7.seqservice.eventFileDirectorySuffixes`	A list of eventFileDirectory path entries to monitor for event files. The value is one or more comma-separated integers.	99 Configured on install to point to 1
`miseq.v7.seqservice.netPathPrefixSearchReplaceSuffixes`	A list of netPathPrefix search and replace entries for transforming Windows to Linux network paths. The value is one or more comma-separated integers.	99 Configured on install to point to 1

Property	Description	Default Value
`miseq.v7.seqservice.runReportViewsVersion`	The current version of the Run Report views in the database. The value 0 represents the state before the views are created. This property is automatically updated by the run report.	0
`miseq.v7.seqservice.ignoreUnmatchedContainerIds`	A flag indicating if event files that cannot be matched to reagent cartridges in Clarity LIMS should be archived after a certain period (true) or continually reprocessed (false).	False To prevent the gls_events file directory from becoming cluttered, it is recommended that the value of this property is set to true
`miseq.v7.seqservice.ignoreUnmatchedContainerIdsWaitDays`	The number of days between when the event is created and the event file is archived.	14
`miseq.v7.seqservice.synchronizationPeriod`	Invocation period in seconds.	60

You can configure support for multiple, identical seqservice.netPathPrefixSearch property values. For more information, see *Configure Multiple Identical netPathPrefixSearch Values* on page 2596

Consumables Installed

Reagent Categories and Kits

Reagent categories or label groups are installed with the Preconfigured Workflow Package (PCWP) workflow configuration slices.

The MiSeq Reagent Kit is included in the Illumina MiSeq Integration.

Control Types

The PhiX v3 control type is included in the Illumina MiSeq Integration.

Container Types

The MiSeq Reagent Cartridge container type is included in the Illumina MiSeq Integration.

All one-dimensional container types with both numeric rows and numeric columns are supported.

Instrument Integration

To make sure that the Illumina instrument warranty remains valid, the instrument integration must be performed and maintained by the Clarity LIMS Support team. To perform this integration, the Support team requires remote access to the system while it is idle.

The following steps are performed by the Clarity LIMS Support team when configuring the sequencing for use with the Illumina MiSeq Integration.

1. Create a directory on the local computer to hold the batch files. These batch files write event files to the network attached storage (NAS) shares.
2. Create a directory on the NAS to hold the event files.
3. Modify Illumina software configuration files to call the batch files that create the event files.
4. Update sequencing service default database properties to match the specifics of the installation.

Rules and Constraints

The Illumina MiSeq Integration operates with the following constraints:

- The reagent cartridge ID must be unique. There should not be multiple reagent cartridge containers in the system with the same name.
- The reagent cartridge ID must be scanned as the reagent cartridge Container Name on the Denature & Dilute (MiSeq v2.0) step.

MiSeq Integration v7.0 Configuration and User Interaction

The Illumina MiSeq Integration Package v7.0 supports the integration of Clarity LIMS to Illumina MiSeq Sequencing Systems.

The integration allows for automated tracking of an Illumina sequencing run in Clarity LIMS. This capability includes tracking sequencing run status, generating run report, and capturing and parsing run statistics. In addition, this integration provides automated generation of a sample sheet file for use with the MiSeq Control Software (MCS).

Overview

The Illumina MiSeq Integration Package v7.0 includes the following features:

- Preconfigured MiSeq Sequencing v2.0 workflow that maps to lab protocol steps and sequencing runs.
- Automated generation of a sample sheet for use with the MiSeq Control Software (MCS).
- Automated tracking of the MiSeq sequencing run and parsing of run statistics into Clarity LIMS, including:
  - Run status and metrics of sequencing run
  - Sequencing run parameters
  - Real-Time Analysis (RTA) v1 run directory location and other run-specific information

This integration also includes a preconfigured Library Prep Validation workflow that is used for validation purposes only. The workflow contains a single-step protocol that models the library prep required to produce normalized libraries that are ready for the MiSeq Sequencing v2.0 workflow. For details, see *MiSeq Integration v7.0 Validation and Troubleshooting* on page 2158.

Depending on the Clarity LIMS version, install the workflows as follows.

- For Clarity LIMS v4 (4.2 and later), install the following workflows via Preconfigured Workflows Package (PCWP) v2:
  - MiSeq Sequencing v2.0 workflow
  - Library Prep Validation v1.1 workflow
- For Clarity LIMS v5 (5.1 and later), install the following workflows via Illumina Preset Protocols (IPP) v1:
  - MiSeq Sequencing v2.0 workflow
  - Library Prep Validation v2.0 workflow
- For Clarity LIMS v5.0.x, contact Illumina Technical Support to upgrade your integration.

The following sections describe the MiSeq Sequencing v2.0 workflow and the integration between Clarity LIMS and the Illumina MiSeq system.

For instructions on validating and troubleshooting the Illumina MiSeq Integration Package, see *MiSeq Integration v7.0 Validation and Troubleshooting* on page 2158.

Prerequisites and Assumptions

Before samples are assigned to the workflow, make sure that the following prerequisites are completed:

- Samples have been run through QC and library prep.
- Samples have been normalized, and the value is captured in a field called Normalized Molarity (nM).
- Reagent labels are attached to samples.
- Samples have been accessioned into Clarity LIMS. For more information on sample accessioning, see *Sample Accessioning* in the Getting Started section of the Clarity LIMS Core Functionality documentation.

Samples can be assigned to the MiSeq Sequencing v2.0 workflow automatically using a routing script or manually from the Projects & Samples dashboard. See *Assign and Process Samples* in the Getting Started section of the Clarity LIMS Core Functionality documentation.

Workflows, Protocols, and Steps

The Illumina MiSeq Integration includes the MiSeq Sequencing v2.0 workflow and the MiSeq Sequencing v2.0 protocol.

The protocol includes the following steps and automations.

Table 449 MiSeq Sequencing v2.0 Protocol Steps and Automations

Step	Automation
1. Library Pooling (MiSeq v2.0)	• Set Next Step - Advance
2. Denature & Dilute (MiSeq v2.0)	• Generate MiSeq SampleSheet • Set Next Step - Advance
3. MiSeq Run (MiSeq v2.0)	• Set Next Step - Advance

Terminology

This integration supports Clarity LIMS v4.2 and later.

As of Clarity LIMS v5, the term user-defined field (UDF) has been replaced with custom field in the user interface. However, the API resource is still called UDF.

There are two types of custom fields:

- Master step fields—Configured on master steps. Master step fields only apply to the following:
  - The master step on which the fields are configured.
  - The steps derived from those master steps.

- Global fields—Configured on entities (eg, submitted sample, derived sample, measurement, etc.). Global fields apply to the entire Clarity LIMS system.

Step 1: Library Pooling (MiSeq v2.0)

In Clarity LIMS v5, the Library Pooling (MiSeq v2.0) step is derived from the Library Pooling v2.0 master step.

In this step, the lab scientist manually places libraries into pools in the Clarity LIMS Placement screen.

Set Next Step - Advance Automation

This automation advances samples to the next step in the protocol. The automation is automatically triggered on exit of the Record Details screen.

Default command line:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-t false \
-h false \
-exp 'nextStep = ::ADVANCE::' \
-log {compoundOutputFileLuid0}"
```

Step UDFs/Custom Fields

The Comment field is configured on the Library Pooling (MiSeq v2.0) step. The Comment field displays on the Record Details screen at run time.

Table 450 Library Pooling (MiSeq v2.0) Step UDF/Custom Field Configuration

Field Name	Field Type	Field Constraints/Options
Comment	Multiline Text	• Required: No • Read Only: No

Global UDFs/Custom Fields

The following table lists the global UDFs/fields that are displayed on the Queue and Ice Bucket screens of the Library Pooling (MiSeq v2.0) step. Most fields display in expanded view only.

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Table 451 Global UDF/Custom Field Configuration for Library Pooling (MiSeq v2.0)

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Configured on Sample in Clarity LIMS v4 and Submitted Sample in Clarity LIMS v5:			
Application	Single-line Text (v4) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: Yes	• TruSeq mRNA sequencing • TruSeq DNA sequencing (large genome de novo) • TruSeq DNA sequencing (large genome reseq) • TruSeq DNA sequencing (small genome de novo) • TruSeq DNA sequencing (small genome reseq) • Nextera DNA sequencing • TruSeq Custom Amplicon sequencing • ChIP-sequencing • Exome sequencing • Mate pair sequencing • Small RNA sequencing
Pooling	Single-line Text (v4) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: Yes	• Yes • No
Read Length	Single-line Text (v4) Text (v5)	• Required: No • Read Only: No	Not applicable
Sequencing Coverage	Single-line Text (v4) Text (v5)	• Required: No • Read Only: No	Not applicable

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Sequencing Method	Single-line Text (v4) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: Yes	Not applicable
Configured on Analyte in Clarity LIMS v4 and Derived Sample in Clarity LIMS v5:			
Normalized Molarity (nM)	Numeric	• Required: No • Read Only: No	Decimal Places Displayed: 2

User Interaction

1. On the Queue screen, do as follows.
   a. Add the samples to the Ice Bucket.
   b. [Optional] In the Add Control Samples panel, add the PhiX v3 control sample to the Ice Bucket.
   c. Select View Ice Bucket.
2. On the Ice Bucket screen, select Begin Work.
3. On the Pool Samples screen, do as follows.
   a. Create a pool of samples by dragging samples into the Pool Creator.
   b. Name the pool or accept the default name (Pool #1).
   c. Select Place Samples.
4. On the Placement screen, do as follows.
   a. Select the pool in the Samples to be Placed area and drag it to the container.
   b. Select Record Details.
5. On the Record Details screen, select Next Steps.
   On the Assign Next Steps screen, next step is already set to Denature and Dilute (MiSeq v2.0).
6. Select Finish Step.

Step 2: Denature and Dilute (MiSeq v2.0)

In this step, pooled libraries are denatured and diluted, and then placed into the reagent cartridge loaded into the MiSeq instrument.

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Generate MiSeq SampleSheet Automation

This automation generates the sample sheet and attaches it to the Denature and Dilute step. Select a button on the Record Details screen to trigger this automation. For details, see *Sample Sheet Generation* on page 2150.

Default command line:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/DriverFileGenerator.jar -i {stepURI:v2} -u {username} -p {password} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/MiSeqSamplesheetv2.csv \
-o {compoundOutputFileLuid1}.csv \
-q true \
-destLIMSID {compoundOutputFileLuid1} \
-l {compoundOutputFileLuid2}"
```

Set Next Step - Advance Automation

This automation advances samples to the next step in the protocol. The automation is automatically triggered by exiting the Record Details screen.

In Clarity LIMS v4, the automation also invokes the setUDF script, which sets the value of the Progress UDF. Clarity LIMS updates this UDF value as samples progress through the workflow steps. The Progress UDF does not display in the Clarity LIMS user interface but does display in the LabLink interface. This feature allows collaborators to see the status of samples.

Default command line (Clarity LIMS v4):

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -u {username} -p {password} \
script:setUDF \
-i {processURI:v2} \
-f 'Progress' \
-t '//input/@uri->//sample/@uri' \
-v 'Library ready for sequencing' \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -u {username} -p {password} \
script:evaluateDynamicExpression \
-i {stepURI:v2} \
-t false \
-h false \
-exp 'nextStep = ::ADVANCE::' \
-log {compoundOutputFileLuid3}"
```

Default command line (Clarity LIMS v5):

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-t false \
-h false \
-exp 'nextStep = ::ADVANCE::' \
-log {compoundOutputFileLuid3}"
```

Step UDFs/Custom Fields

The fields configured on the Denature & Dilute (MiSeq v2.0) step display on the Record Details screen, in the Step Data table. These fields are manually populated at run time (see *User Interaction* on page 2147).

The values are then used to generate the sample sheet. For details on the sample generation functionality, see *Sample Sheet Generation* on page 2150.

Table 452 Denature & Dilute (MiSeq v2.0) Step UDF/Custom Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Adapter	Single-line Text (v4) Text (v5)	• Required: No • Read Only: No	Not applicable
Assay	Single-line Text (v4) Text (v5)	• Required: No • Read Only: No	Not applicable
Comment	Multiline Text	• Required: No • Read Only: No	Not applicable
Control CAT Manifest	Single-line Text (v4) Text (v5)	• Required: No • Read Only: No	Not applicable
Custom CAT Manifest	Single-line Text (v4) Text (v5)	• Required: No • Read Only: No	Not applicable

For Research Use Only. Not for use in diagnostic procedures.

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Custom Primers	Single-line Text (v4) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: No	• None (Default) • Read 1 • Index • Read 2 • Read 1, Index • Read 1, Index, Read 2 • Read 1, Read 2 • Index, Read 2
Description	Single-line Text (v4) Text (v5)	• Required: No • Read Only: No	Not applicable
Experiment Name	Single-line Text (v4) Text (v5)	• Required: Yes • Read Only: No	Not applicable
FilterPCRDuplicates	Checkbox (v4) Toggle Switch (v5)	• Required: No • Read Only: No	• Default: None Set
GenomeFolder	Single-line Text (v4) Text (v5)	• Required: No • Read Only: No	Not applicable
MinQScore	Numeric	• Required: No • Read Only: No	• Range From: 0 to 100 • Decimal Places Displayed: 2
OnlyGenerateFASTQ	Checkbox (v4) Toggle Switch (v5)	• Required: No • Read Only: No	• Default: None Set
Read 1 Cycles	Numeric (v4) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 251 (Default) • 151 • 101 • 76 • 51 • Range From: 0 to 1000 • Decimal Places Displayed: 0

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Read 2 Cycles	Numeric (v4) Numeric Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: Yes	• 251 • 151 • 101 • 76 • 51 • Range From: 0 to 1000 • Decimal Places Displayed: 0
SiteReports *Hidden field, does not display on Record Details screen*	Single-line Text (v4) Text (v5)	• Required: No • Read Only: No	Not applicable
StitchReads *Only for GenerateFASTQ, Amplicon DS and TruSeqAmplicon workflows*	Checkbox (v4) Toggle Switch (v5)	• Required: No • Read Only: No	• Default: None Set
Use submitted sample details for Genome Folder location	Checkbox (v4) Toggle Switch (v5)	• Required: No • Read Only: No	• Default: None Set
VariantCaller	Single-line Text (v4) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: No	• GATK (Default) • Somatic • Starling • None
Workflow	Single-line Text (v4) Text Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: No	• Assembly • Custom Amplicon • Enrichment • GenerateFASTQ • LibraryQC • Metagenomics • PCR Amplicon • Resequencing

Groups of Defaults/Presets

The following groups of defaults/presets are configured for Denature & Dilute (MiSeq v2.0) step UDFs/master step custom fields:

Resequencing

Field	Configuration
Custom Primers	None
FilterPCRDuplicates	Yes
GenomeFolder	Required
OnlyGenerateFastQ	No
Read 1 Cycles	251
StitchReads	None Set
Use submitted sample details for Genome Folder location	No
Workflow	Resequencing

Custom Amplicon

Field	Configuration
Custom CAT Manifest	Required
Custom Primers	None
FilterPCRDuplicates	Yes
GenomeFolder	Required
OnlyGenerateFastQ	No
Read 1 Cycles	251
StitchReads	None Set
Use submitted sample details for Genome Folder location	No
Workflow	Custom Amplicon

PCR Amplicon

Field	Configuration
Custom CAT Manifest	Required
Custom Primers	None
FilterPCRDuplicates	Yes
GenomeFolder	Required
OnlyGenerateFastQ	No
Read 1 Cycles	251
StitchReads	None Set
Use submitted sample details for Genome Folder location	No
Workflow	PCR Amplicon

Enrichment

Field	Configuration
Custom CAT Manifest	Required
Custom Primers	None
FilterPCRDuplicates	Yes
GenomeFolder	Required
OnlyGenerateFastQ	No
Read 1 Cycles	251
StitchReads	None Set
Use submitted sample details for Genome Folder location	No
Workflow	Enrichment

Library QC

Field	Configuration
Custom Primers	None
FilterPCRDuplicates	Yes
GenomeFolder	Required

Field	Configuration
OnlyGenerateFastQ	No
Read 1 Cycles	251
StitchReads	None Set
Use submitted sample details for Genome Folder location	No
Workflow	LibraryQC

Metagenomics

Field	Configuration
Custom Primers	None
FilterPCRDuplicates	Yes
OnlyGenerateFastQ	No
Read 1 Cycles	251
StitchReads	None Set
Use submitted sample details for Genome Folder location	No
Workflow	Metagenomics

GenerateFastQ

Field	Configuration
Custom Primers	None
FilterPCRDuplicates	Yes
OnlyGenerateFastQ	Yes
Read 1 Cycles	251
StitchReads	None Set
Use submitted sample details for Genome Folder location	No
Workflow	GenerateFastQ

Assembly

Field	Configuration
Custom Primers	None
FilterPCRDuplicates	Yes
OnlyGenerateFastQ	No
Read 1 Cycles	251
StitchReads	None Set
Use submitted sample details for Genome Folder location	No
Workflow	Assembly

Global UDFs/Custom Fields

The following table shows the global UDFs/fields that are configured to display on the Queue, Ice Bucket, and Record Details screens of the Denature & Dilute (MiSeq v2.0) step.

Table 453 Global UDF/Custom Field Configuration for Denature & Dilute (MiSeq v2.0) step

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Configured on Sample in LIMS v4 and Submitted Sample in LIMS v5:			
Progress *(LIMS v4) Displays in LabLink Collaborations Interface only*	Single-line Text (v4) Text (v5)	• Required: No • Read Only: No • Custom Entries: Yes	Not applicable
Read Length *Displays on Queue & Ice Bucket screens*	Single-line Text (v4) Text (v5)	• Required: No • Read Only: No	Not applicable
Sequencing Method *Displays on Queue & Ice Bucket screens*	Single-line Text (v4) Text Dropdown (v5)	• Required: No • Read Only: No • Custom Entries: Yes	• Single Read • Paired End Read • Indexed Single Read • Indexed Paired End Read

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Configured on Analyte in LIMS v4 and Derived Sample in LIMS v5:			
Final Loading Concentration *Displays on Record Details screen*	Numeric (v4) Numeric Dropdown (v5)	• Required: Yes • Read Only: No • Custom Entries: Yes	• 225 • 400

Step File Placeholders

Placeholders for the following files are configured on the Record Details screen of the Denature & Dilute (MiSeq v2.0) step:

- Lab Tracking Form (manually generated)
- MiSeq SampleSheet (autogenerated)
- MiSeq SampleSheet Generation Log (autogenerated)
- Log File (autogenerated)

Table 454 Sample Sheet Files and Placeholders Generated by Denature & Dilute (MiSeq v2.0) Step

Item	Description
Lab Tracking Form placeholder	This placeholder in Clarity LIMS allows the lab scientist to attach a lab-specific tracking form to the step manually.
MiSeq SampleSheet	This CSV file is automatically generated by Clarity LIMS for use with the MiSeq system. The file can be opened as a text file or an Excel spreadsheet.

For Research Use Only. Not for use in diagnostic procedures.

Item	Description
MiSeq SampleSheet Generation Log	This log file is automatically generated by Clarity LIMS. The log file captures any errors that Clarity LIMS might encounter when generating the sample sheet.
Log File	This log file is automatically generated by Clarity LIMS. The log file captures the status of the EvaluateDynamicExpression script that is invoked by the Set Next Step - Advance automation.

User Interaction

1. On the Queue screen, add the pool to the Ice Bucket, and then select View Ice Bucket.
2. On the Ice Bucket screen, select Begin Work.
3. On the Placement screen, do as follows.
   a. Scan the barcode of the MiSeq reagent cartridge into the MiSeq Reagent Cartridge field.
   b. Place the pool of samples into the reagent cartridge.
   c. Select Record Details.
4. On the Record Details screen, do as follows.
   a. Under Reagent Lot Tracking, select the reagent lot used in the step.
   If the reagent lot is not listed, add/activate the lot on the Reagents and Controls screen.
   b. Populate the fields in the Step Details table, as needed. Workflow and Read 1 Cycles are required fields.
   c. In the Sample Details table, enter the Final Loading Concentration. Select from the following preset options or enter a different value.
      - 225 (for PCR-free workflows)
      - 400 (for Nano workflows)
   d. Select Generate MiSeq SampleSheet.
   Clarity LIMS generates the sample sheet and attaches it and a log file to placeholders in the Files area of the Record Details screen.
   e. Download the files and validate their format and content.

For Research Use Only. Not for use in diagnostic procedures.

5. Select Next Steps.
   On the Assign Next Steps screen, samples are already assigned to the MiSeq Run (MiSeq v2.0) step.
6. Select Finish Step.

See also *Sample Sheet Generation* on page 2150.

Step 3: MiSeq Run (MiSeq v2.0)

Set Next Step - Advance Automation

This automation advances samples to the next step in the protocol. The automation is automatically triggered by exiting the Record Details screen.

Default command line:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-t false \
-h false \
-exp 'nextStep = ::ADVANCE::' \
-log {compoundOutputFileLuid5}"
```

Step UDFs/Custom Fields

There are 18 fields configured on the MiSeq Run (MiSeq v2.0) step. These fields display on the Record Details screen at run time.

The read-only fields are automatically populated at the end of the run.

Table 455 MiSeq Run (MiSeq v2.0) Step UDF/Custom Field Configuration

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Chemistry	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable
Comment	Multiline Text	• Required: No • Read Only: No	Not applicable
Experiment Name	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable

For Research Use Only. Not for use in diagnostic procedures.

Field Name	Field Type	Field Constraints/Options	Preset Values/Additional Options and Drop-Down Items
Finish Date	Date	• Required: No • Read Only: Yes	Not applicable
Flow Cell ID	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable
Flow Cell Version	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable
Index 1 Read Cycles	Numeric	• Required: No • Read Only: Yes	Decimal Places Displayed: 0
Index 2 Read Cycles	Numeric	• Required: No • Read Only: Yes	Decimal Places Displayed: 0
Output Folder	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable
PR2 Bottle ID	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable
Read 1 Cycles	Numeric	• Required: No • Read Only: Yes	Decimal Places Displayed: 0
Read 2 Cycles	Numeric	• Required: No • Read Only: Yes	Decimal Places Displayed: 0
Reagent Cartridge ID	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable
Reagent Cartridge Part #	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable
Run ID	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable
Run Type	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable
Status	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable
Workflow	Single-line Text (v4) Text (v5)	• Required: No • Read Only: Yes	Not applicable

Global UDFs/Custom Fields

There are several sample and measurement global UDFs/fields that are displayed on the Record Details screen of the MiSeq Run (MiSeq v2.0) step. These fields are autopopulated at the end of the sequencing run.

For more information, see *User Interaction* on page 2150 and *Sequencing Results Parsing* on page 2151.

Step File Placeholders

Placeholders for the following files are configured on the Record Details screen of the MiSeq Run (MiSeq v2.0) step:

- Illumina Run Report (autogenerated)
- Link to Run Folder (autogenerated)
- Run Parameters (autogenerated)
- Run Info (autogenerated)
- Lab Tracking Form (manually generated)
- Log File (autogenerated)

For details, see *Generated and Captured Files* on page 2151.

User Interaction

1. On the Queue screen, add the pool to the Ice Bucket, and then select View Ice Bucket.
2. On the Ice Bucket screen, select Begin Work.
3. On the Record Details screen, the fields in the table are read-only. After the run completes, the integration automatically performs the following actions:
   - Populates the fields.
   - Attaches files to the Illumina Run Report, Link to Run Folder, Run Parameters, and Run Info placeholders.
   - Populates the fields in the Sample Details table.
4. Select Next Steps.
   On the Assign Next Steps screen, the next step is already set to Mark protocol as complete.
5. Select Finish Step.

Sample Sheet Generation

Sample sheet generation occurs in the Denature & Dilute (MiSeq v2.0) step. This step places samples on the container loaded in the system.

The default configuration provides only the Generate MiSeq SampleSheet automation. This automation uses the Template File Generator (`DriverFileGenerator.jar`) and a template file to generate a CSV format file for use with the MiSeq Control Software (MCS).

The sample sheet content is determined by the fields on the Record Details screen of the step in the Step Data table. The values entered into these fields are used to populate the sample sheet.

To customize the template used to create the sample sheet, you can insert additional columns.

There are additional details available:

- See *Illumina Instrument Sample Sheets (NGS v5.17 & later)* on page 2542, which provides a sample template that you can download and customize for the lab.
- For instructions on customizing the template, see *Creating Template Files* on page 2454.

Sequencing Results Parsing

The MiSeq Run (MiSeq v2.0) step records information for the flow cell lane and generates a report summarizing the results. In addition, run parameters, run info, and a link to the run folder are automatically captured.

Generated and Captured Files

The following table describes the run information files, reports, placeholders, and links that Clarity LIMS automatically generates or captures during a sequencing run.

Table 456 Run Information Generated or Captured by MiSeq Run (MiSeq v2.0) Step

Item	Description
Run Info Run Parameters	These XML files are automatically captured by Clarity LIMS from the run folder of the system. They include the key run parameters that are parsed out into step UDFs/custom fields in Clarity LIMS.
Link to Run Folder	This link is the path to the network run folder where the data that was captured from the system during the run is stored. The link is automatically generated by Clarity LIMS.
Illumina Run Report	This report provides key information about the run and the samples on the flow cell. The report it automatically generated by Clarity LIMS. Information includes the flow cell ID, run directory location, and primary analysis metrics for the sequencing run. Information is summarized per flow cell lane for the entire run and individual reads for paired-end runs. These metrics are compared against the per lane averages of the sequencing run. The per lane averages are calculated using metrics from the last five sequencing runs. Any values outside of one standard deviation are highlighted.

For Research Use Only. Not for use in diagnostic procedures.

Item	Description
Lab Tracking Form placeholder	This placeholder in Clarity LIMS allows you to attach a lab-specific tracking form to the step manually.

Metadata

The following list shows metadata that Clarity LIMS automatically captures from the Illumina sequencing software as part of a sequencing run. This information is gathered from various run result files and events.

- Chemistry
- Experiment Name (entered in software)
- Finish Date (run completion date)
  - If the End Run event contains a date in the format YYYY-MM-DD, Finish Date is set to the date in the event file.
  - If the End Run event does not contain a date or the date is in the wrong format, Finish Date is set to the date when the event file is processed.
- Flow Cell ID
- Flow Cell Version
- Index 1 Read Cycles (intended Index cycles)
- Index 2 Read Cycles (intended Index cycles)
- Output Folder (run folder root)
- PR2 Bottle ID
- Reagent Cartridge ID
- Reagent Cartridge Part #
- Read 1 Cycles
- Read 2 Cycles
- Run ID (the unique run ID)
- Run Type
- Status (current status of the sequencing run on the instrument)
- Workflow

Primary Analysis Metrics

The following table lists the Real-Time Analysis (RTA) primary analysis metrics Clarity LIMS automatically captures and records per read, for samples in each flow cell lane. These metrics are captured upon run completion and are stored as UDFs/custom fields in the Sample Details table on the

Record Details screen.

To see both per read and per lane metrics, expand the output.

Table 457 RTA Primary Analysis Metrics Captured by MiSeq Run (MiSeq v2.0) Step

Per Read LIMS Field Name *(Stored on derived sample/analyte input to the step)*	Per Lane LIMS Field Name *(Stored in measurement placeholders in the Sample Details table on the Record Details screen)*
% Aligned R1	% Aligned R1
% Aligned R2	% Aligned R2
% Bases >=Q30 R1	% Bases >=Q30 R1
% Bases >=Q30 R2	% Bases >=Q30 R2
% Error Rate R1	% Error Rate R1
% Error Rate R2	% Error Rate R2
Not applicable	% Phasing R1
Not applicable	% Phasing R2
Not applicable	% Prephasing R1
Not applicable	% Prephasing R2
Not applicable	%PF R1
Not applicable	%PF R2
Not applicable	Cluster Density (K/mm^2) R1
Not applicable	Cluster Density (K/mm^2) R2
Intensity Cycle 1 R1	Intensity Cycle 1 R1
Intensity Cycle 1 R2	Intensity Cycle 1 R2
Not applicable	Reads PF (M) R1
Not applicable	Reads PF (M) R2
Yield PF (Gb) R1	Yield PF (Gb) R1
Yield PF (Gb) R2	Yield PF (Gb) R2

How It Works

1. The sequencing service runs on the Clarity LIMS server. The service detects event files that instrument RTA produces as the run progresses. The event files let the service know where to find the run data.
2. As the run data are written out and the End Run event is detected, the following events occur:

- The data are matched to the step based on the reagent cartridge ID that was entered or scanned on the Denature & Dilute (MiSeq v2.0) step.
- Read-only field values on the Record Details screen are populated accordingly.

3. When the service has finished processing the end run event and updating the fields in Clarity LIMS, the sequencing service generates the report and attaches it to the step.

User Interaction

1. Manually start the step, and then leave the step in progress until the sequencing service has finished processing the run and the run report is generated and attached.
2. After the run has completed, verify the following items:
   - The read-only fields on the Record Details screen are populated.
   - The Illumina Run Report is generated and attached.
   - The Link to Run Folder is generated and attached.
   - The Run Parameters and Run Info files are attached.

   Leave the step as in progress until the Illumina Run Report has been attached to the step in Clarity LIMS.
3. [Optional] In the Files section, upload the Lab Tracking Form.
4. Select Next Steps.
   On the Assign Next Steps screen, the next step is already set to Mark protocol as complete. The Log File is attached when the next step for samples has been assigned by the Next Step - Advance automation.
5. Select Finish Step.

Components Installed

This integration requires components installed with the Preconfigured Workflow Package (PCWP).

The IlluminaMiSeq Integration Package v7.0 RPM installs the scripts and files listed in the following table.

Table 458 Illumina MiSeq Integration Package v7.0 Scripts and Files Installed

Files Installed	Location & Description
`configure_extensions_miseq_sequencingservice-v7.sh`	/opt/gls/clarity/config/ Script that installs the service properties in the database.
`log4j.xml`	/opt/gls/clarity/extensions/Illumina_MiSeq/v7/SequencingService/conf File containing the settings for logging the sequencing jar.

For Research Use Only. Not for use in diagnostic procedures.

Files Installed	Location & Description
`miseq-sequencing-v7.jar`	/opt/gls/clarity/extensions/Illumina_MiSeq/v7/SequencingService Jar file containing API-based Clarity LIMS extensions used for capturing run results and report generation.
InterOp libraries	/opt/gls/clarity/extensions/Illumina_MiSeq/v7/lib Illumina shared library for parsing InterOp data files.
`MiSeqSamplesheetv2.csv` (Installed by the PCWP or IPP)	/opt/gls/clarity/extensions/conf/driverfiletemplates Template file used for file generation.

Database Properties Installed

The Illumina MiSeq Integration v7.1 also installs several additional database properties, each with the 99 suffix appended to their name. These properties are intended for use by the Clarity LIMS support team in automated validation tests and are not listed in the table.

Sequencing runs are matched using the reagent cartridge ID and the base name of the sequencing step, MiSeq Run (MiSeq v2.0).

Do not change the base name. The sequencing service that captures sequencing run results expects this name. The base name is stored in the `sequenceProcessBaseName` database property. If the name is changed without the database property being updated, the reagent cartridge ID <-> sequencing step base name matching system fails.

If you need to rename the step, for example to add a version number, modify the text after the base name portion. This part of the name is not used in the matching system. For example, change MiSeq Run (MiSeq v2.0) to MiSeq Run (MiSeq v2.0) v2.

Table 459 Database Properties Installed with the Illumina MiSeq Integration Package

Property	Description	Default Value
`miseq.v7.seqservice.sequenceProcessBaseName`	Sequencing process type/master step base display name. Partial matching is used to look up the process type/master step.	MiSeq Run (MiSeq v2.0)

For Research Use Only. Not for use in diagnostic procedures.

Property	Description	Default Value
`miseq.v7.seqservice.eventFileDirectory.1`	A network location monitored for event files, eg, `/mnt/illumina/gls_events/`	/mnt/gls_events
`miseq.v7.seqservice.netPathPrefixSearch.1`	The network directory prefix contained in the event file.	\\nas\network\run_data
`miseq.v7.seqservice.netPathPrefixReplace.1`	The mapped network directory mount name on the server used to access the run data directory, eg, `/mnt/network/data`	/mnt/run_data
`miseq.v7.seqservice.eventFileDirectorySuffixes`	A list of eventFileDirectory path entries to monitor for event files. The value is one or more comma-separated integers.	99 Configured on install to point to 1
`miseq.v7.seqservice.netPathPrefixSearchReplaceSuffixes`	A list of netPathPrefix search and replace entries for transforming Windows to Linux network paths. The value is one or more comma-separated integers.	99 Configured on install to point to 1

Property	Description	Default Value
`miseq.v7.seqservice.runReportViewsVersion`	The current version of the Run Report views in the database. The value 0 represents the state before the views are created. This property is automatically updated by the run report.	0
`miseq.v7.seqservice.ignoreUnmatchedContainerIds`	A flag indicating if event files that cannot be matched to reagent cartridges in Clarity LIMS should be archived after a certain period (true) or continually reprocessed (false).	False To prevent the gls_events file directory from becoming cluttered, it is recommended that the value of this property is set to true
`miseq.v7.seqservice.ignoreUnmatchedContainerIdsWaitDays`	The number of days between when the event is created and the event file is archived.	14
`miseq.v7.seqservice.synchronizationPeriod`	Invocation period in seconds.	60

You can configure support for multiple, identical seqservice.netPathPrefixSearch property values. For more information, see *Configure Multiple Identical netPathPrefixSearch Values* on page 2596

Consumables Installed

Reagent Categories and Kits

Reagent categories or label groups are installed with the Preconfigured Workflow Package (PCWP) workflow configuration slices.

The MiSeq Reagent Kit is included in the Illumina MiSeq Integration.

Control Types

The PhiX v3 control type is included in the Illumina MiSeq Integration.

Container Types

The MiSeq Reagent Cartridge container type is included in the Illumina MiSeq Integration.

All one-dimensional container types with both numeric rows and numeric columns are supported.

Instrument Integration

To make sure that the Illumina instrument warranty remains valid, the instrument integration must be performed and maintained by the Clarity LIMS Support team. To perform this integration, the Support team requires remote access to the system while it is idle.

The following steps are performed by the Clarity LIMS Support team when configuring the sequencing for use with the Illumina MiSeq Integration.

1. Create a directory on the local computer to hold the batch files. These batch files write event files to the network attached storage (NAS) shares.
2. Create a directory on the NAS to hold the event files.
3. Modify Illumina software configuration files to call the batch files that create the event files.
4. Update sequencing service default database properties to match the specifics of the installation.

Rules and Constraints

The Illumina MiSeq Integration operates with the following constraints:

- The reagent cartridge ID must be unique. There should not be multiple reagent cartridge containers in the system with the same name.
- The reagent cartridge ID must be scanned as the reagent cartridge Container Name on the Denature & Dilute (MiSeq v2.0) step.

MiSeq Integration v7.0 Validation and Troubleshooting

The following sections explain how to validate the installation of the Illumina MiSeq Integration Package v7.0.

The validation involves the following processes:

- Running samples through the Library Prep Validation workflow. The workflow contains a single-step protocol that models the library prep required to produce normalized libraries. At the end of the step, the normalized libraries automatically advance to the MiSeq Sequencing v2.0 workflow.
- Running normalized libraries through the MiSeq Sequencing v2.0 workflow. This process validates the following information:
  - Automated generation of a sample sheet for use with the MiSeq Control Software (MCS).
  - Automated tracking of the MiSeq sequencing run and parsing of run statistics into Clarity LIMS, including the following information:
    - Run status and metrics of sequencing run
    - Sequencing run parameters
    - Real-Time Analysis (RTA) v1 run directory location and other run-specific information

The version of Library Prep Validation workflow depends on whether the workflow is delivered by PreConfigured Workflows Package (PCWP) or Illumina Preset Protocols (IPP).

- Library Prep Validation v1.1 (PCWP)
- Library Prep Validation v2.0 (IPP)

Activate Workflow, Create Project, Add and Assign Samples

The following steps prepare Clarity LIMS to run samples through the Library Prep Validation and MiSeq Sequencing v2.0 workflows.

1. In the Clarity LIMS Configuration area, activate the Library Prep Validation and MiSeq Sequencing v2.0 workflows.
   - In Clarity LIMS v4, select the workflow and select Activate.
   - In Clarity LIMS v5, select the workflow and change the Workflow Status toggle to Activate.
2. On the Projects & Samples screen, create a project, and then add samples to the project.
3. Assign the samples to the Library Prep Validation workflow.

Library Prep Validation Protocol

This single-step protocol models the library prep required to produce normalized libraries that are ready for the MiSeq Sequencing v2.0 workflow.

Run Library Prep Validation Step

1. In Lab View, locate the Library Prep Validation protocol. Samples are queued for the Library Prep Validation step. Select the step to proceed to the Queue screen.
2. On the Queue screen, add the samples to the Ice Bucket, and then select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.

4. On the Placement screen, do as follows.
   a. In the container field in the Placed Samples area, scan or enter the barcode of the destination container.
   b. Select samples from the Samples to be Placed area and drag them to the container wells on the right. Repeat until all samples are placed.
   c. Select Add Labels.
5. On the Add Labels screen, do as follows.
   a. To assign labels, select a label and drag it to a container well. Repeat as needed.
   b. Select Record Details.
6. On the Record Details screen, do as follows.
   a. Select all the samples in the Sample Details table.
   b. In the Sequencing Instrument drop-down list, select MiSeq.
   c. Select Apply to set this value for all selected samples.
   d. Select Next Steps.
   On the Assign Next Steps screen, the next step is automatically set to Remove from Workflow. This setting is required by the Routing script automation.
7. Select Finish Step.
   The Routing script automation is triggered. Samples are routed to the first step in the MiSeq Sequencing v2.0 protocol—Library Pooling (MiSeq v2.0).

MiSeq Sequencing v2.0 Protocol

Run Library Pooling (MiSeq v2.0)

1. In Lab View, locate the MiSeq Sequencing v2.0 protocol. Samples are queued for the Library Pooling (MiSeq v2.0) step. Select the step to proceed to the Queue screen.
2. On the Queue screen, do as follows.
   a. Add the samples to the Ice Bucket.
   b. In the Add Control Samples panel, add the PhiX v3 control sample to the Ice Bucket.
   c. Select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Pool Samples screen, do as follows.
   a. Create a pool of samples by dragging samples into the Pool Creator.
   b. Name the pool or accept the default name (Pool #1).
   c. Select Place Samples.
5. On the Placement screen, do as follows.
   a. Select the pool from the Samples to be Placed area and drag it to the container.

b. Select Record Details.

6. On the Record Details screen, select Next Steps.
   On the Assign Next Steps screen, next step is automatically set to Denature and Dilute (MiSeq v2.0).
7. Select Finish Step.

Run Denature and Dilute (MiSeq v2.0)

1. In Lab View, locate the MiSeq Sequencing v2.0 protocol. The pooled samples are queued for the Denature and Dilute (MiSeq v2.0) step. Select the step to proceed to the Queue screen.
2. On the Queue screen, add the pool to the Ice Bucket, and then select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Placement screen, do as follows.
   a. Scan the MiSeq reagent cartridge barcode into the MiSeq Reagent Cartridge field.
   b. Place the pool of samples into the reagent cartridge.
   c. Select Record Details.
5. On the Record Details screen, do as follows.
   a. In Reagent Lot Tracking, select the reagent lot used in the step.
      If the reagent lot is not listed, add/activate the lot on the Reagents and Controls screen.
   b. Populate the fields in the Step Details table, as needed. Workflow, Experiment Name, and Read 1 Cycles are required fields.
   c. In the Sample Details table, enter the Final Loading Concentration. Select from the following preset options or enter a different value.
      - 225 (for PCR-free workflows)
      - 400 (for Nano workflows)
   d. Select Generate MiSeq SampleSheet.
      Clarity LIMS generates the sample sheet and attaches it and a log file to placeholders in the Files area of the Record Details screen.
   e. Download the files and validate the format and content.
6. Select Next Steps.
   On the Assign Next Steps screen, samples are automatically assigned to the MiSeq Run (MiSeq v2.0) step.
7. Select Finish Step.

Run MiSeq Run (MiSeq v2.0)

1. In Lab View, locate the MiSeq Sequencing v2.0 protocol. The pool of samples is queued for the MiSeq Run (MiSeq v2.0) step. Select the step to proceed to the Queue screen.

2. On the Queue screen, add the pool to the Ice Bucket, and then select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Record Details screen, the fields are read-only. When the run completes, the integration automatically performs the following actions:
   - Populates the fields.
   - Attaches files to the Illumina Run Report, Link to Run Folder, Run Parameters, and Run Info placeholders.
   - Populates the fields in the Sample Details table. See *MiSeq Integration v7.0 Configuration and User Interaction* on page 2133 for details.

   The Log File is attached after the next step for samples is assigned by the Next Step - Advance automation. Make sure the Illumina Run Report has been attached before continuing.
5. Select Next Steps.
   On the Assign Next Steps screen, the next step is automatically set to Mark protocol as complete.
6. Select Finish Step.

Validating Creation of Event Files

This validation checks the following information:

- The destination path is correctly configured.
- The instrument computer can access and write to the destination path.
- There are no syntax errors in the Clarity LIMS batch file.

Use the following steps to confirm that event files are created by the batch file in the destination path. The steps assume that the default configuration has been successfully imported.

1. In `C:\gls`, double-click the batch file (`gls_event_mcs_rta.bat` or `gls_event_mcs_rta_lrm.bat`, depending on MiSeq version).
2. Confirm that an empty event file appears in the configured DESTINATION_PATH.

Manual execution of the batch file produces an output resembling the following example:

```
Filename:
event-EndRun-07295667.txt
Contents:
cycleNumber =
runFolder =
netFolder =
readType =
eventType = EndRun
softwareType = MCS
```


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Troubleshooting

If an automation trigger does not appear to run the corresponding scripts, see the following articles in the Clarity LIMS API & Database documentation:

- *Troubleshooting Automated Informatics*
- *Troubleshooting EPP / Automation*

If an error occurs that does not provide direction on how to proceed, confirm the version of the installed MiSeq Integration Package. To confirm the version, run the following command from the server console:

```
rpm -qa | grep -i miseq
```

If the error is related to data parsing, retrieving run results data, or report values not appearing as expected, review the `MiseqIntegrator.log` file. The file is located at `/opt/gls/clarity/extensions/Illumina_MiSeq/v7/SequencingService`.

Additional troubleshooting information for this integration is provided in the *Illumina Instrument Integrations FAQ* on page 2598.

If you are unable to resolve the issue, contact the Clarity LIMS Support team.

Illumina MiSeq Integration 6.0.1 Release Notes

Overview: Bugfix to enable upgrade validation

Bugs resolved in this release:

- Fixed a bug that prevented MiSeq v5 service from running concurrently with the MiSeq v6 service. This fix enables customers validating upgrades from an older version, by allowing both versions of the Sequencing Service to work side by side.

Illumina MiSeq Integration v6.0 Configuration and User Interaction

The Illumina MiSeq Integration Package v6.0 supports the integration of Clarity LIMS to Illumina MiSeq instruments.

The integration allows for automated tracking of an Illumina sequencing run in Clarity LIMS. This includes tracking instrument run status, generating run report, and capturing and parsing run statistics. In addition, this integration provides automated generation of a sample sheet file for use with the MiSeq Control Software (MCS).

- This guide describes the integration between Clarity LIMS and the Illumina MiSeq system. It includes information about protocols and automations, configuration options, installed components, and rules and constraints.
- For instructions on validating and troubleshooting the Illumina MiSeq Integration Package, refer to *Illumina MiSeq Integration v6.0 Validation and Troubleshooting* on page 2192.

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Overview

The Illumina MiSeq Integration Package v6.0 includes:

- Preconfigured MiSeq Sequencing v1.0 workflow that maps to lab protocol steps and instrument runs.
- Automated generation of a sample sheet for use with the MiSeq Control Software (MCS). Automated tracking of the MiSeq sequencing run and parsing of run statistics into the LIMS, including:Run status and metrics of sequencing run
  - Sequencing run parameters
  - Real Time Analysis v1 (RTA) run directory location and other run specific information

Information on these features is provided in this guide.

Note that this integration also includes a preconfigured Library Prep Validation v1.0 workflow, used for validation purposes only. The workflow contains a single-step protocol that models the library prep required to produce normalized libraries that are ready for the MiSeq Sequencing v1.0 workflow. For details, refer to *Illumina MiSeq Integration v6.0 Validation and Troubleshooting* on page 2192.

Prerequisites and Assumptions

It is assumed that samples enter the MiSeq Sequencing v1.0 workflow as normalized libraries, and have had reagent labels attached. That is, before they are assigned to the workflow:

- Samples have been accessioned into the LIMS.
- Samples have been run through QC and library prep.
- Samples have been normalized, and the value is captured in a field called Normalized Molarity (nM).

For details on sample accessioning, refer to the following sections in the Clarity LIMS Core Functionality documentation (under the Samples section):

- *Sample Accessioning*
- *Using Sample Lists to Upload and Modify Samples*

Samples can be assigned to the MiSeq Sequencing v1.0 workflow automatically (using a routing script), or manually from the Projects and Samples dashboard (refer to *Assigning Samples to Workflows* in the Clarity LIMS Core Functionality documentation).

Workflows, Protocols, and Steps

The Illumina MiSeq Integration includes the MiSeq Sequencing v1.0 workflow, which contains a single protocol of the same name.

The MiSeq Sequencing v1.0 protocol includes the following steps:

- Library Pooling (MiSeq v1.0)

- Denature and Dilute (MiSeq v1.0)
- MiSeq Run (MiSeq v1.0)

The following image shows the protocol and steps as displayed to the lab scientist working in Clarity LIMS Lab View.



Step Automations

The following table lists the step automations configured on the MiSeq Sequencing v1.0 protocol steps. Automation details are provided in *Configuration Details* on page 2165.

Table 460 MiSeq Sequencing v1.0 Protocol Step Automations

Step	Automation
1. Library Pooling (MiSeq v1.0)	Set Next Step - Advance
2. Denature and Dilute (MiSeq v1.0)	Generate MiSeq SampleSheet Set Next Step - Advance
3. MiSeq Run (MiSeq v1.0)	Set Next Step - Advance

Configuration Details

The sections below describe the configuration installed with the Illumina MiSeq Integration. The Illumina MiSeq Integration Package v6.0 supports BaseSpace Clarity LIMS v4.2.x and v5.x.

As of Clarity LIMS v5, the term user-defined field (UDF) has been replaced with custom field in the user interface. However, the API resource is still called UDF.

There are two types of custom fields:

- Master step fields—Configured on master steps. Master step fields only apply to the following:
  - The master step on which the fields are configured.
  - The steps derived from those master steps.
- Global fields—Configured on entities (eg, submitted sample, derived sample, measurement, etc.). Global fields apply to the entire Clarity LIMS system.

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Step 1: Library Pooling (MiSeq v1.0)

Note that in LIMS v5, the Library Pooling (MiSeq v1.0) step is derived from the Library Pooling v1.0 master step.

In this step, user manually places libraries into pools.

Automations

One automation is configured on this step: Set Next Step - Advance

Figure 25 Automation configuration on Library Pooling (MiSeq v1.0) step - LIMS v4.2

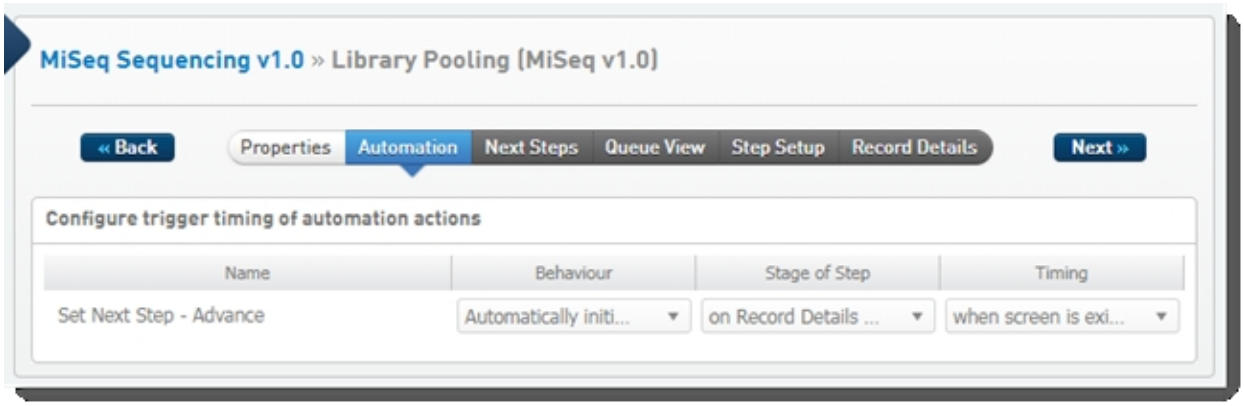
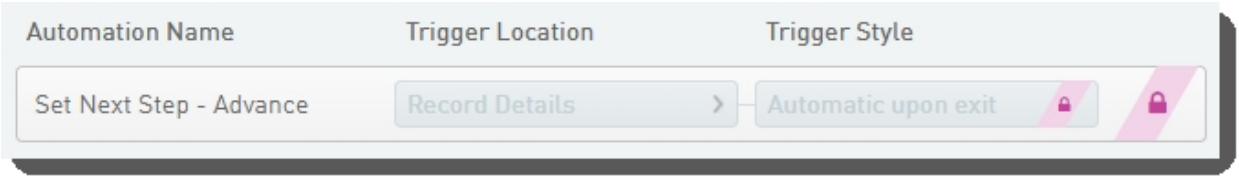


Figure 26 Automation configuration on Library Pooling (MiSeq v1.0) step - LIMS v5 and later



Set Next Step - Advance Automation

Automatically triggered on exit of the Record Details screen, this automation advances samples to the next step in the protocol.

Default command line:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression \ -t false \ -h false \ -exp 'nextStep = ::ADVANCE::' \ -log {compoundOutputFileLuid0}"
```

Step UDFs/Custom Fields

There is one field configured on the Library Pooling (MiSeq v1.0) step. This field displays on the Record Details screen at run time.

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The field is shown below in the Clarity LIMS v5 Configuration area (Custom Fields > Master Step Fields tab). Refer to the following table for LIMS v4.2 and LIMS v5 field configuration details.

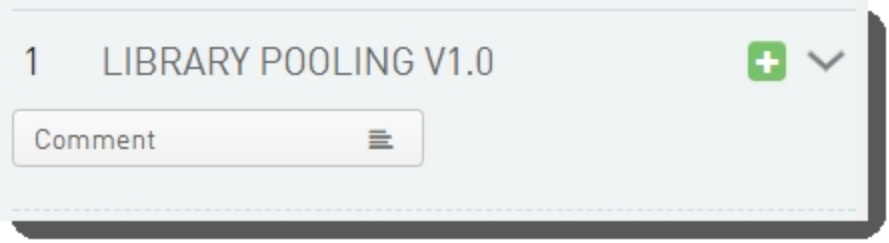


Table 461 Library Pooling (MiSeq v1.0) step UDF / custom field configuration

Field Name	Field Type	Field Constraints / Options
Comment	Multi-line Text	Not applicable

Global UDFs/custom fields

The following table lists the global UDFs / fields that are configured to display on the Queue and Ice Bucket screens of the Library Pooling (MiSeq v1.0) step. Note that the majority of these fields display in expanded view only.

Table 462 Global UDF / Custom Field Configuration for Library Pooling (MiSeq v1.0)

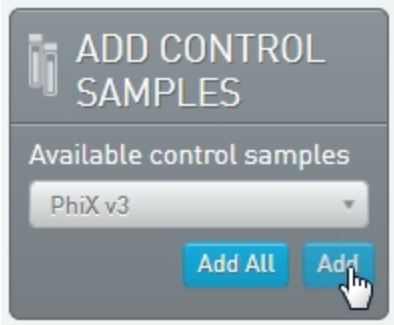
Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
Configured on Sample in LIMS v4.2 and Submitted Sample in LIMS v5:			
Application	Single-line text (v4.2) Text Dropdown (v5)	Custom Entries	Presets • TruSeq mRNA sequencing • TruSeq DNA sequencing (large genome de novo) • TruSeq DNA sequencing (large genome re-seq) • TruSeq DNA sequencing (small genome de novo) • TruSeq DNA sequencing (small genome re-seq) • Nextera DNA sequencing • TruSeq Custom Amplicon sequencing • ChIP-sequencing • Exome sequencing • Mate pair sequencing • Small RNA Sequencing

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Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
Pooling	Single-line text (v4.2) Text Dropdown (v5)	Custom Entries	Presets • Yes • No
Read Length	Single-line text (v4.2) Text (v5)	Not applicable	Not applicable
Sequencing Coverage	Single-line text (v4.2) Text (v5)	Not applicable	Not applicable
Sequencing Method	Single-line text (v4.2) Text Dropdown (v5)	Custom Entries	Presets • Single Read • Paired End Read • Indexed Single read • Indexed Paired End Read
Configured on Analyte in LIMS v4.2 and Derived Sample in LIMS v5:			
Normalized Molarity (nM)	Numeric	Not applicable	Decimal places displayed = 2

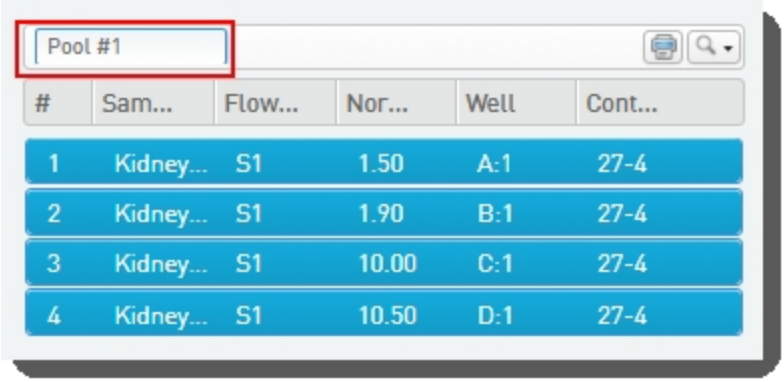
User Interaction

1. On the Queue screen, add the samples to the Ice Bucket.
    - In the Add Control Samples panel, add the PhiX v3 control sample to the Ice Bucket.



    - Select View Ice Bucket.
2. On the Ice Bucket screen, select Begin Work.
3. On the Pool Samples screen, create a pool of samples by dragging samples into the Pool Creator.
    - Name the pool, or accept the default name - Pool #1.

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- Select Place Samples.

4. On the Placement screen, select the pool in the Samples to be Placed area on the left and drag it over to the container on the right.
    - Select Record Details.
5. On the Record Details screen, select Next Steps.
6. On the Assign Next Steps screen, next step is already set to Denature and Dilute (MiSeq v1.0). Select Finish Step.

Step 2: Denature and Dilute (MiSeq v1.0)

In this step, pooled libraries are denatured and diluted and placed into the reagent cartridge that will be loaded into the MiSeq instrument.

Automations

The following automations are configured on this step:

- Generate MiSeq SampleSheet
- Set Next Step - Advance

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Figure 27 Automation configuration on Denature and Dilute (MiSeq v1.0) step - LIMS v4.2

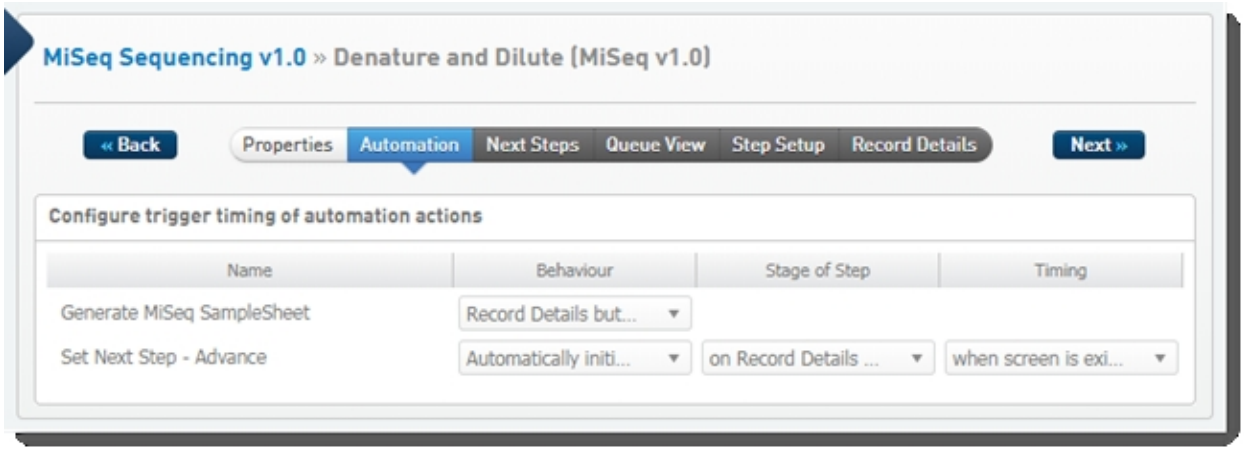
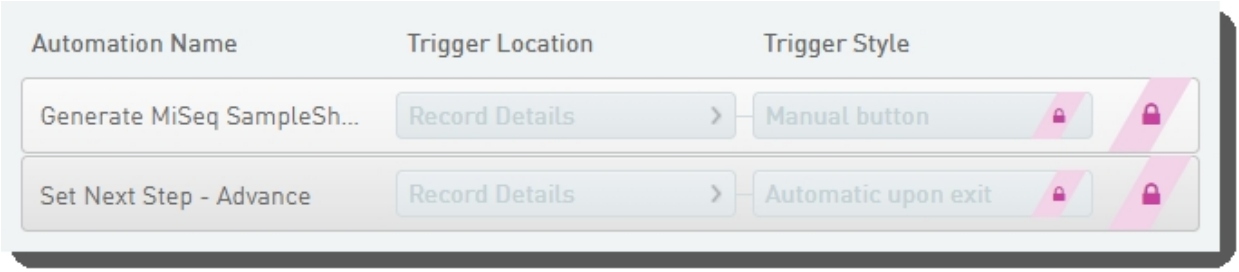


Figure 28 Automation configuration on Denature and Dilute (MiSeq v1.0) step - LIMS v4.2



Generate MiSeq SampleSheet Automation

Triggered when the user selects a button on the Record Details screen, this automation generates generates the sample sheet and attaches it to the step. For details, refer to *Sample Sheet Generation* on page 2183.

Default command line:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -u {username} -p {password} \
script:driver_file_generator \ -i {processURI:v2} \ -t
/opt/gls/clarity/extensions/conf/driverfiletemplates/MiSeqSamplesheet.csv \ -o
{compoundOutputFileLuid1}.csv \ -q true \ -destLIMSID {compoundOutputFileLuid1}
\ -l {compoundOutputFileLuid2}"
```

Set Next Step - Advance Automation

Automatically triggered on exit of the Record Details screen, this automation advances samples to the next step in the protocol.

In LIMS v4.2, the automation also invokes the `setUDF` script, which sets the value of the Progress UDF. Clarity LIMS updates this UDF value as samples progress through the workflow steps. The Progress UDF does not display in the LIMS user interface, but it does display in the LabLink Collaborations Interface so that collaborators can see the status of their samples.

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Default command line (LIMS v4.2):

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -u {username} -p {password} \ script:setUDF \ -i {processURI:v2} \ -f 'Progress' \ -t '//input/@uri->//sample/@uri' \ -v 'Library ready for sequencing' \ && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -u {username} -p {password} \ script:evaluateDynamicExpression \ -i {stepURI:v2} \ -t false \ -h false \ -exp 'nextStep = ::ADVANCE::' \ -log {compoundOutputFileLuid3}"
```

Default command line (LIMS v5):

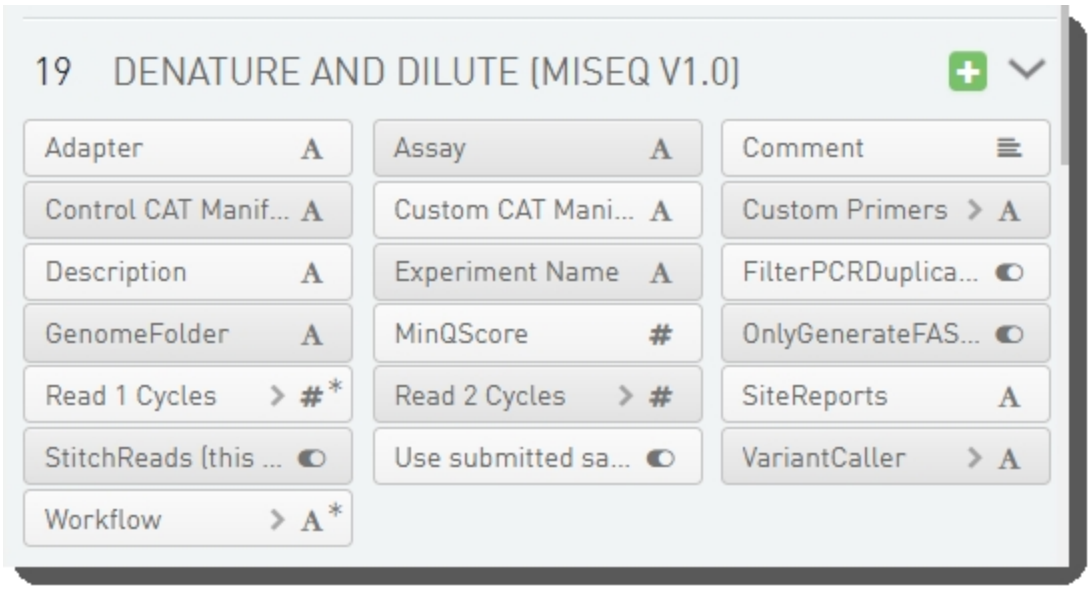
```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression \ -t false \ -h false \ -exp 'nextStep = ::ADVANCE::' \ -log {compoundOutputFileLuid3}"
```

Step UDFs/Custom Fields

There are 19 fields configured on the Denature and Dilute (MiSeq v1.0) step. These are shown below in the Clarity LIMS v5 Configuration area (Custom Fields > Master Step Fields tab).

At run time, these fields display on the Record Details screen, in the Step Data table. The user manually populates the fields (refer to *User Interaction* on page 2178) and the values are then used to generate the sample sheet. For details on the sample generation functionality, refer to *Sample Sheet Generation* on page 2183.

Refer to the following table for LIMS v4.2 and LIMS v5 field configuration details.



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Table 463 Denature and Dilute (MiSeq v1.0) Step UDF / Custom Field Configuration

Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
Adapter	Single-line Text (v4.2) Text (v5)	Not applicable	Not applicable
Assay	Single-line Text (v4.2) Text (v5)	Not applicable	Not applicable
Comment	Multi-line Text	Not applicable	Not applicable
Control CAT Manifest	Single-line Text (v4.2) Text (v5)	Not applicable	Not applicable
Custom CAT Manifest	Single-line Text (v4.2) Text (v5)	Not applicable	Not applicable
Custom Primers	Single-line Text (v4.2) Text Dropdown (v5)	Not applicable	Presets • None (Default) • Read 1 • Index • Read 2 • Read 1, Index • Read 1, Index, Read 2 • Read 1, Read 2 • Index, Read 2
Description	Single-line Text (v4.2) Text (v5)	Not applicable	Not applicable
Experiment Name	Single-line Text (v4.2) Text (v5)	Not applicable	Not applicable
FilterPCRDuplicates	Check Box (v4.2) Toggle Switch (v5)	Not applicable	Default • None Set

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Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
GenomeFolder	Single-line Text (v4.2) Text (v5)	Not applicable	Not applicable
MinQScore	Numeric	Not applicable	Range = 0–1000 Decimal places displayed =2
OnlyGenerateFASTQ	Check Box (v4.2) Toggle Switch (v5)	Not applicable	Default • None Set
Read 1 Cycles	Numeric (v4.2) Numeric Dropdown (v5)	Custom Entries	Presets • 251 (Default) • 151 • 101 • 76 • 51 Range = 0–1000 Decimal places displayed = 0
Read 2 Cycles	Numeric (v4.2) Numeric Dropdown (v5)	Custom Entries	Presets • 251 • 151 • 101 • 76 • 51 Range = 0–1000 Decimal places displayed = 0
SiteReports (Hidden field, does not display on Record Details screen)	Single-line Text (v4.2) Text (v5)	Not applicable	Not applicable
StitchReads (only for GenerateFASTQ, Amplicon-DS and TruSeqAmplicon workflows)	Check Box (v4.2) Toggle Switch (v5)	Not applicable	Default • None Set

Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
Use submitted sample details for Genome Folder location	Check Box (v4.2) Toggle Switch (v5)	Not applicable	Default • None Set
VariantCaller	Single-line Text (v4.2) Text Dropdown (v5)	Not applicable	Presets • GATK (Default) • Somatic • Starling • None
Workflow	Single-line Text (v4.2) Text Dropdown (v5)	Not applicable	Presets • Assembly • Custom Amplicon • Enrichment • GenerateFASTQ • LibraryQC • Metagenomics • PCR Amplicon • Resequencing

Groups of Defaults/Presets

The following group of defaults / presets are configured for Denature and Dilute (MiSeq v1.0) step UDFs / master step custom fields:

- Resequencing
  - Custom Primers: None
  - FilterPCRDuplicates: Yes
  - GenomeFolder: Required
  - OnlyGenerateFastQ: No
  - Read 1 Cycles: 251
  - StitchReads: None Set
  - Use submitted sample details for Genome Folder location: No
  - Workflow: Resequencing
- Custom Amplicon
  - Custom CAT Manifest: Required
  - Custom Primers: None
  - FilterPCRDuplicates: Yes

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  - GenomeFolder: Required
  - OnlyGenerateFastQ: No
  - Read 1 Cycles: 251
  - StitchReads: None Set
  - Use submitted sample details for Genome Folder location: No
  - Workflow: Custom Amplicon
- PCR Amplicon
  - Custom CAT Manifest: Required
  - Custom Primers: None
  - FilterPCRDuplicates: Yes
  - GenomeFolder: Required
  - OnlyGenerateFastQ: No
  - Read 1 Cycles: 251
  - StitchReads: None Set
  - Use submitted sample details for Genome Folder location: No
  - Workflow: PCR Amplicon
- Enrichment
  - Custom CAT Manifest: Required
  - Custom Primers: None
  - FilterPCRDuplicates: Yes
  - GenomeFolder: Required
  - OnlyGenerateFastQ: No
  - Read 1 Cycles: 251
  - StitchReads: None Set
  - Use submitted sample details for Genome Folder location: No
  - Workflow: Enrichment
- Library QC
  - Custom Primers: None
  - FilterPCRDuplicates: Yes
  - GenomeFolder: Required
  - OnlyGenerateFastQ: No
  - Read 1 Cycles: 251
  - StitchReads: None Set
  - Use submitted sample details for Genome Folder location: No

  - Workflow: LibraryQC
- Metagenomics
  - Custom Primers: None
  - FilterPCRDuplicates: Yes
  - OnlyGenerateFastQ: No
  - Read 1 Cycles: 251
  - StitchReads: None Set
  - Use submitted sample details for Genome Folder location: No
  - Workflow: Metagenomics
- GenerateFastQ
  - Custom Primers: None
  - FilterPCRDuplicates: Yes
  - OnlyGenerateFastQ: Yes
  - Read 1 Cycles: 251
  - StitchReads: None Set
  - Use submitted sample details for Genome Folder location: No
  - Workflow: GenerateFastQ
- Assembly
  - Custom Primers: None
  - FilterPCRDuplicates: Yes
  - OnlyGenerateFastQ: No
  - Read 1 Cycles: 251
  - StitchReads: None Set
  - Use submitted sample details for Genome Folder location: No
  - Workflow: Assembly

Global UDFs/Custom Fields

The following table shows the global UDFs / fields that are configured to display on the Queue, Ice Bucket and Record Details screens of the Denature and Dilute (MiSeq v1.0) step.

Table 464 Global UDF / Custom Field Configuration for Denature and Dilute (MiSeq v1.0) Step

Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
Configured on Sample in LIMS v4.2 and Submitted Sample in LIMS v5:			

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Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
Progress (LIMS v4.2) Displays in LabLink Collaborations Interface only	Single-line Text (v4.2) Text (v5)	Custom Entries	Not applicable
Read Length Displays on Queue and Ice Bucket screens	Single-line Text (v4.2) Text (v5)	Not applicable	Not applicable
Sequencing Method Displays on Queue and Ice Bucket screens	Single-line Text (v4.2) Text Dropdown (v5)	Custom Entries	Presets • Single Read • Paired End Read • Indexed Single Read • Indexed Paired End Read
Configured on Analyte in LIMS v4.2 and Derived Sample in LIMS v5:			
Final Loading Concentration Displays on Record Details screen	Numeric (v4.2) Numeric Dropdown (v5)	Required Field Custom Entries	Presets • 225 • 400

Step File Placeholders

Placeholders for the following files are configured on the Record Details screen of the Denature and Dilute (MiSeq v1.0) step:

- Lab Tracking Form (manually generated)
- MiSeq Samplesheet (auto-generated)
- MiSeq Samplesheet Generation Log (auto-generated)
- Log File (auto-generated)

Table 465 Sample Sheet Files and Placeholders Generated by Denature and Dilute (MiSeq v1.0) Step

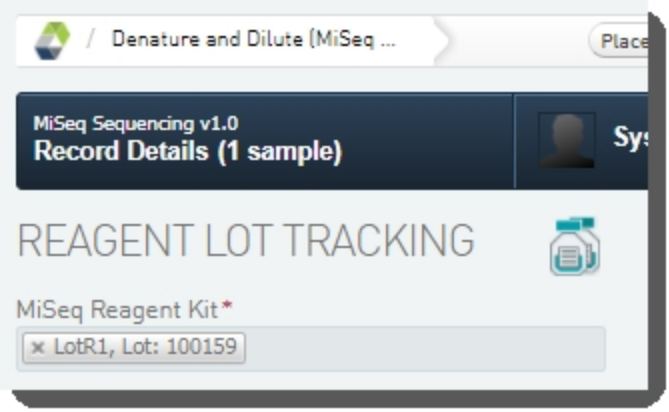
Item	Description
Lab Tracking Form placeholder	This placeholder in the LIMS allows the user to manually attach a lab-specific tracking form to the step.

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Item	Description
MiSeq Sample Sheet	This CSV file is automatically generated by the LIMS for use with the MiSeq instrument. May be opened as a text file, or as an MS Excel spreadsheet.
Sample Sheet Generation Log	Automatically generated by the LIMS, this log file captures any errors that the LIMS may encounter when generating the sample sheet.
Log File	Automatically generated by the LIMS, this log file captures the status of the `EvaluateDynamicExpression` script that is invoked by the Set Next Step - Advance automation.

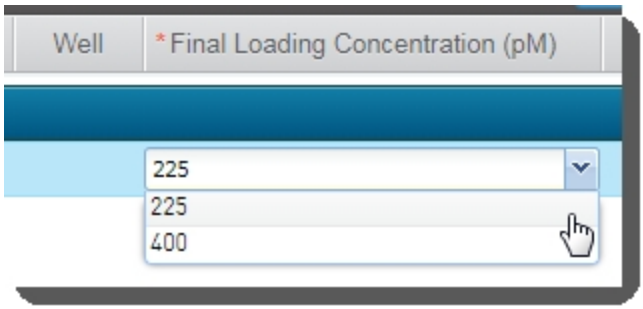
User Interaction

1. On the Queue screen, add the pool to the Ice Bucket and select View Ice Bucket.
2. On the Ice Bucket screen, select Begin Work.
3. On the Placement screen, scan the barcode of the MiSeq reagent cartridge into the MiSeq Reagent Cartridge field.
    - Place the pool of samples into the reagent cartridge.
    - Select Record Details.
4. On the Record Details screen, under Reagent Lot Tracking, select the reagent lot used in the step. You may need to add/activate the lot on the Reagents and Controls screen.



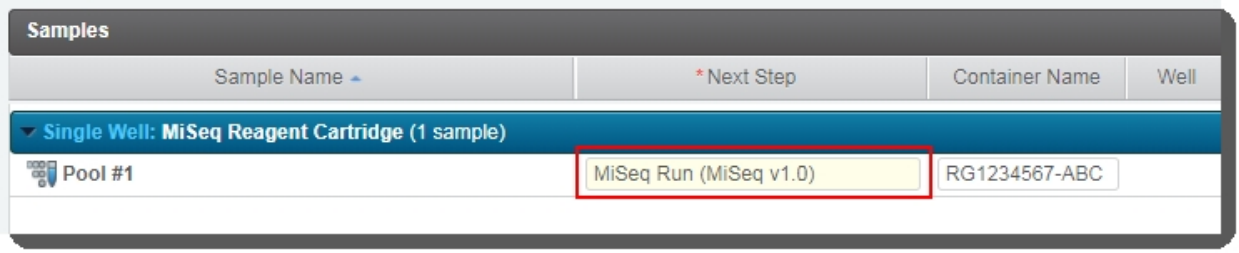
    - Populate the fields in the Step Details table, as appropriate. Note that Workflow and Read 1 Cycles are required fields.
    - In the Sample Details table, enter the Final Loading Concentration. You may select from the two preset options: 225 (for PCR-free workflows) or 400 (for Nano workflows), or enter a different value.

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- Select Generate MiSeq SampleSheet.
- The LIMS generates the samplesheet and attaches it and a log file to placeholders in the Files area of the Record Details screen. Download the files and validate their format and content.
- Select Next Steps.

5. On the Assign Next Steps screen, samples are already assigned to the MiSeq Run (MiSeq v1.0)step.



6. Select Finish Step.

Refer to *Sample Sheet Generation* on page 2183.

Step 3: MiSeq Run (MiSeq v1.0)

Automations

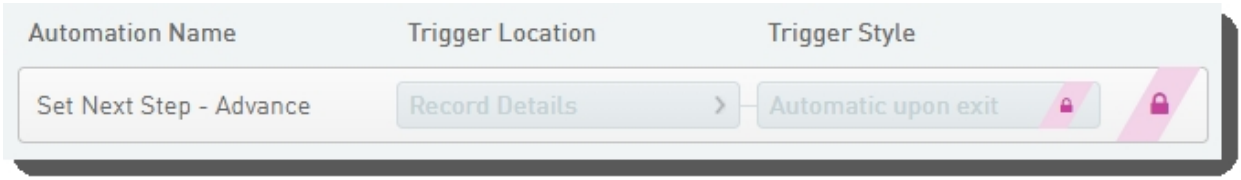
One automation is configured on this step: Set Next Step - Advance

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Figure 29 Automation configuration on MiSeq Run (MiSeq v1.0) step - LIMS v4.2



Figure 30 Automation configuration on MiSeq Run (MiSeq v1.0) step - LIMS v4.2



Set Next Step - Advance Automation

Automatically triggered on exit of the Record Details screen, this automation advances samples to the next step in the protocol.

Default command line:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression \ -t false \ -h false \ -exp 'nextStep = ::ADVANCE::' \ -log {compoundOutputFileLuid5}"
```

Step UDFs/Custom Fields

There are 18 fields configured on the MiSeq Run (MiSeq v1.0) step. These fields display on the Record Details screen at run time.

Some of the field values are completed by the user running the step, the remaining fields are automatically populated at the end of the run.

The fields are shown below in the BaseSpace Clarity LIMS v5 Configuration area (Custom Fields > Master Step Fields tab). Refer to the following table for LIMS v4.2 and LIMS v5 field configuration details.

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Table 466 MiSeq Run (MiSeq v1.0) Step UDF / Custom Field Configuration

Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
Chemistry	Single-line Text (v4.2) Text (v5)	Read Only	Not applicable
Comment	Multi-line Text	Not applicable	Not applicable
Experiment Name	Single-line Text (v4.2) Text (v5)	Read Only	Not applicable
Finish Date	Date	Read Only	Not applicable
Flow Cell ID	Single-line Text (v4.2) Text (v5)	Read Only	Not applicable
Flow Cell Version	Single-line Text (v4.2) Text (v5)	Read Only	Not applicable
Index 1 Read Cycles	Numeric	Read Only	Decimal Places Displayed: 0
Index 2 Read Cycles	Numeric	Read Only	Decimal Places Displayed: 0

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Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options and Dropdown Items
Output Folder	Single-line Text (v4.2) Text (v5)	Read Only	Not applicable
PR2 Bottle ID	Single-line Text (v4.2) Text (v5)	Read Only	Not applicable
Read 1 Cycles	Numeric	Read Only	Decimal Places Displayed: 0
Read 2 Cycles	Numeric	Read Only	Decimal Places Displayed: 0
Reagent Cartridge ID	Single-line Text (v4.2) Text (v5)	Read Only	Not applicable
Reagent Cartridge Part #	Single-line Text (v4.2) Text (v5)	Read Only	Not applicable
Run ID	Single-line Text (v4.2) Text (v5)	Read Only	Not applicable
Run Type	Single-line Text (v4.2) Text (v5)	Read Only	Not applicable
Status	Single-line Text (v4.2) Text (v5)	Read Only	Not applicable
Workflow	Single-line Text (v4.2) Text (v5)	Read Only	Not applicable

Global UDFs/Custom Fields

There are a number of sample and measurement global UDFs / fields that are configured to display on the Record Details screen of the MiSeq Run (MiSeq v1.0) step. These fields are auto-populated at the end of the sequencing run.

For more information, refer to *User Interaction* on page 2183and *Sequencing Results Parsing* on page 2184.

Step file Placeholders

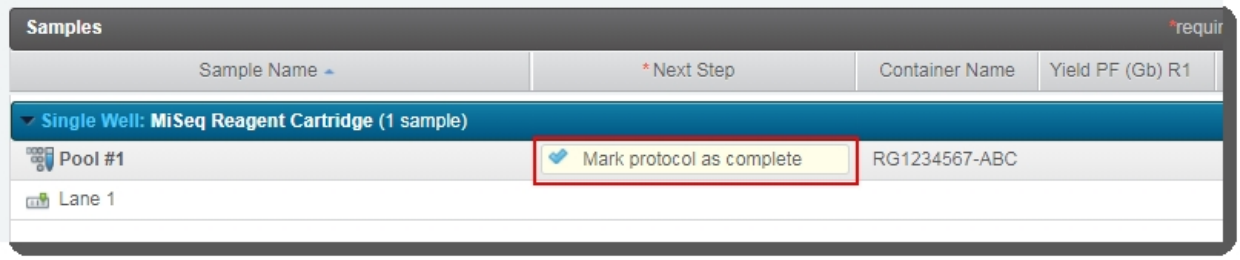
Placeholders for the following files are configured on the Record Details screen of the MiSeq Run (MiSeq v1.0) step:

- Illumina Run Report (auto-generated)
- Link to Run Folder (auto-generated)
- Run Parameters (auto-generated)
- Run Info (auto-generated)
- Lab Tracking Form (manually generated)
- Log File (auto-generated)

For details, refer to *Generated and Captured Files* on page 2184.

User Interaction

1. On the Queue screen, add the pool to the Ice Bucket and select View Ice Bucket.
2. On the Ice Bucket screen, select Begin Work.
3. On the Record Details screen, the fields in the table are read-only. Once the run completes, the integration will automatically populate the fields.
    - Attach files to the Illumina Run Report, Link to Run Folder, Run Parameters, and Run Info placeholders.
    - Populate the fields in the Sample Details table.
4. Select Next Steps.
5. On the Assign Next Steps screen, the next step is already set to Mark protocol as complete.



6. Select Finish Step.

Sample Sheet Generation

Sample sheet generation occurs on the step prior to the sequencing run – Denature and Dilute (MiSeq v1.0), which is the step where samples are placed on the container that will be loaded in the instrument.

The out-of-the-box configuration provides one automation, Generate MiSeq SampleSheet, which uses the Template File Generator (`DriverFileGenerator.jar`) and a template file to generate a CSV format file for use with the MiSeq Control Software (MCS).

The sample sheet content is determined by the fields that display on the Record Details screen of the step (in the Step Data table), and the values that the user enters into these fields.

You can customize the template used to create the sample sheet, inserting adding additional columns to meet the needs of your lab.

For details:

- Refer to the *Illumina Instrument Sample Sheets (NGS v5.17 & later)* on page 2542, which provides a sample template that you can download and customize for your lab.
- For instructions on customizing the template, refer to *Creating Template Files* on page 2454.

Sequencing Results Parsing

The MiSeq Run (MiSeq v1.0) step records information for the flow cell lane and generates a report summarizing the results. In addition, run parameters, run info, and a link to the run folder are automatically captured.

Generated and Captured Files

The following table describes the run information files, reports, placeholders, and links that the LIMS automatically generates or captures during a sequencing run.

Table 467 Run Information Generated or Captured by MiSeq Run (MiSeq v1.0) Step

Item	Description
Run Info Run Parameters	These XML files are automatically captured by Clarity LIMS from the instrument's run folder. They include the key run parameters, many of which are parsed out into key step UDFs.
Link to Run Folder	Automatically generated by Clarity LIMS, this is a link to the network run folder where the data that was captured from the instrument during the run is stored.
Illumina Run Report	Automatically generated by Clarity LIMS, this report provides key information about the run and the samples on the flow cell. Information includes the flow cell ID, run directory location, and primary analysis metrics for the instrument – summarized per flow cell lane for the entire run, and individual reads in the case of paired-end runs. These metrics are compared against the instrument's per lane averages, calculated using metrics from the last 5 sequencing runs. Any values outside of 1 standard deviation are highlighted.
Lab Tracking Form placeholder	This placeholder in the LIMS allows the user to manually attach a lab-specific tracking form to the step.

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Metadata

Listed below is the metadata that the LIMS automatically captures from the Illumina sequencing software as part of a sequencing run. This information is gathered from various run result files and events. (To see how these fields are configured in the LIMS, refer to *Step UDFs/Custom Fields* on page 2180.)

- Chemistry
- Experiment Name – entered in software
- Finish Date* – run completion date
- Flow Cell ID
- Flow Cell Version
- Index 1 Read Cycles – intended Index cycles
- Index 2 Read Cycles – intended Index cycles
- Output Folder – run folder root
- PR2 Bottle ID
- Reagent Cartridge ID
- Reagent Cartridge Part #
- Read 1 Cycles
- Read 2 Cycles
- Run ID – the unique run ID
- Run Type
- Status – current status of the sequencing run on the instrument
- Workflow

Finish Date is populated as follows:

- If the End Run event contains a date in the format YYYY-MM-DD, Finish Date will be set to the date in the event file.
- If the End Run event does not contain a date or the date is in the wrong format, Finish Date will be set to the date when the event file is processed.

Primary Analysis Metrics

The following table lists the Real Time Analysis (RTA) primary analysis metrics the LIMS automatically captures and records, per read, for samples in each flow cell lane. These metrics are captured upon run completion and are stored as UDFs/custom fields in the Record Details screen's Sample Details table.

Users can see both per read and per lane metrics by expanding the output.

Table 468 RTA Primary Analysis Metrics captured by MiSeq Run (MiSeq v1.0) Step

Per Read LIMS Field Name (stored on derived sample/analyte input to the step)	Per Lane LIMS Field Name (stored in measurement placeholders in Record Details screen's Sample Details table)
% Aligned R1	% Aligned R1
% Aligned R2	% Aligned R2
% Bases >=Q30 R1	% Bases >=Q30 R1
% Bases >=Q30 R2	% Bases >=Q30 R2
% Error Rate R1	% Error Rate R1
% Error Rate R2	% Error Rate R2
Not applicable	% Phasing R1
Not applicable	% Phasing R2
Not applicable	% Prephasing R1
Not applicable	% Prephasing R2
Not applicable	%PF R1
Not applicable	%PF R2
Not applicable	Cluster Density (K/mm^2) R1
Not applicable	Cluster Density (K/mm^2) R2
Intensity Cycle 1 R1	Intensity Cycle 1 R1
Intensity Cycle 1 R2	Intensity Cycle 1 R2
Not applicable	Reads PF (M) R1
Not applicable	Reads PF (M) R2
Yield PF (Gb) R1	Yield PF (Gb) R1
Yield PF (Gb) R2	Yield PF (Gb) R2

How It Works

1. The sequencing service runs on the Clarity LIMS server. The service detects event files that the instrument software (RTA) produces as the run progresses; this in turn lets the service know where to find the run data.
2. As the run data is written out and the End Run event is detected: The data is matched to the step based on the reagent cartridge ID, which was entered/scanned in by the user on the Denature and Dilute (MiSeq v1.0) step.
    - Read-only field values on the Record Details screen are populated accordingly.

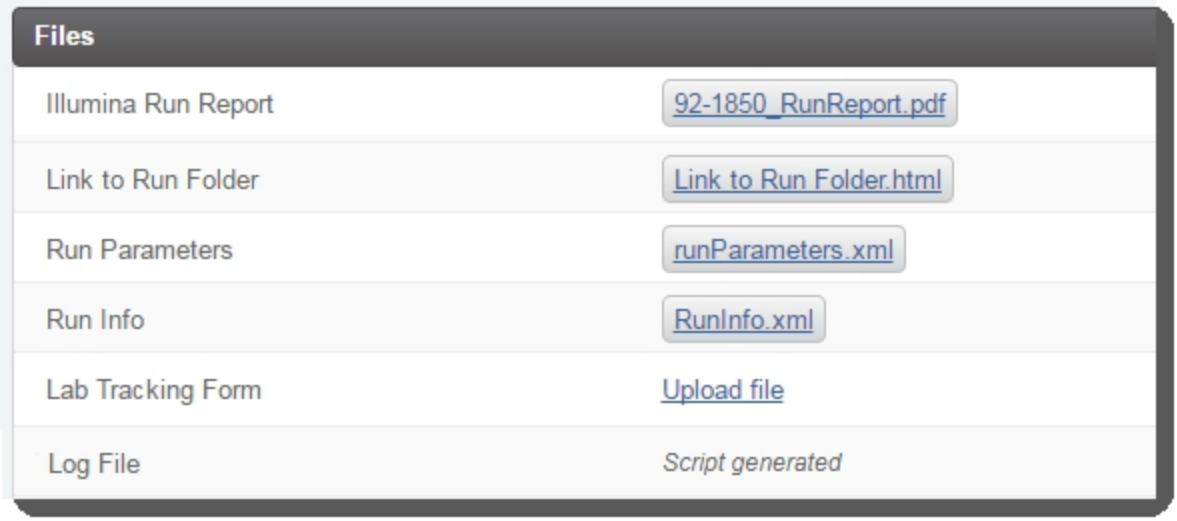
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3. When it has finished processing the end run event and updating the fields in the LIMS, the sequencing service generates the report and attaches it to the step.

User Interaction

1. The user manually starts the step and leaves it in progress until the sequencing service has finished processing the run and the run report is generated and attached.
2. On the Record Details screen, the read-only field values are automatically populated as the sequencer runs.
3. Once the run has completed:The read-only fields shown in the screenshot above are populated.
    - The Illumina Run Report is generated and attached.
    - The Link to Run Folder is generated and attached.
    - The Run Parameters and Run Info files are attached.
4. [Optional] In the Files section, the user uploads the Lab Tracking Form.

Figure 31 Record Details Screen (Files Section) of MiSeq Run (MiSeq v1.0) Step



User must not complete the step until the Illumina Run Report has been attached to the step in Clarity LIMS.

5. The user completes the step.
   Note that the Log File is attached once the next step for samples has been assigned by the Next Step - Advance automation.

Components installed

This integration requires installation of the Preconfigured Workflow Package (PCWP). For details, refer to the Release Notes.

The Illumina MiSeq Integration Package v6.0 RPM installs the scripts and files listed in Table 10. Additional component details are provided in the sections following the table.

Table 469 Illumina MiSeq Integration Package v6.0 - Scripts and Files Installed

Files Installed	Location and Description
configure_extensions_miseq_sequencingservice.sh	*/opt/gls/clarity/config/* Script that installs the service properties in the database
log4j.xml	*/opt/gls/clarity/extensions/miseq/SequencingService/conf* File containing the settings for the sequencing jar's logging.
miseq-sequencing.jar	*/opt/gls/clarity/extensions/miseq/SequencingService* Jar file containing API-based LIMS extensions used for capturing run results and report generation.
InterOp libraries	*/opt/gls/clarity/extensions/miseq/lib* Illumina shared library for parsing InterOp data files.
MiSeqSamplesheet.csv (Installed by the PCWP)	*/opt/gls/clarity/extensions/miseq/Templates* Template file used for file generation.

Support for Multiple seqservice.netPathPrefixSearch Property Values

It is possible to configure support for multiple, identical seqservice.netPathPrefixSearch property values. For details, refer to *Configure Multiple Identical netPathPrefixSearch Values* on page 2596.

Database Properties Installed

The following table lists the database properties installed with Illumina MiSeq Integration v6.0.

Note that several additional properties, each with the '99' suffix appended to their name, are also installed. These are intended for use by the BaseSpace Clarity LIMS support team in automated validation tests and are not listed in the table.

Constraints

Sequencing runs are matched using the reagent cartridge ID and the sequencing step's base name – MiSeq Run (MiSeq v1.0).

Do not change this name – it is expected by the sequencing service that captures instrument run results. The base name is stored in the `sequenceProcessBaseName` database property (refer to the following table). If this name is changed without the database property being updated, the 'reagent cartridge ID <-> sequencing step base name' matching system will fail.

If required, you may modify the step name by editing or adding to the text after the base name portion as this is not used in the matching system. For example, you could change MiSeq Run (MiSeq v1.0) to MiSeq Run (MiSeq v1.0) v2

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Table 470 Database Properties Installed with the Illumina MiSeq Integration Package

Property	Description	Default Value
miseq.v6.seqservice.sequenceProcessBaseName *(installed from the PCWP)*	Sequencing process type / master step base display name. Partial matching is used to look up the process type / master step.	MiSeq Run (MiSeq v1.0)
miseq.v6.seqservice.eventFileDirectory.1	A network location monitored for event files. E.g., /mnt/illumina/gls_events/'	/mnt/gls_events
miseq.v6.seqservice.netPathPrefixSearch.1	The network directory prefix contained in the event file - most likely in Windows format.	\\nas\network\run_data
miseq.v6.seqservice.netPathPrefixReplace.1	The mapped network directory mount name on the server used to access the run data directory. Eg. /mnt/network/data'	/mnt/run_data

Property	Description	Default Value
miseq.v6.seqservice.eventFileDirectorySuffixes	A list of eventFileDirectory path entries to monitor for event files. The value is one or more comma separated integers.	99 Configured on install to point to "1"
miseq.v6.seqservice.netPathPrefixSearchReplaceSuffixes	A list of netPathPrefix search and replace entries for transforming Windows to Linux network paths. The value is one or more comma-separated integers.	99 Configured on install to point to "1"
miseq.v6.seqservice.runReportViewsVersion	The current version of the Run Report views in the database. The value 0 represents the state before the views are created. This property is automatically updated by the run report.	0

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Property	Description	Default Value
miseq.v6.seqservice.ignoreUnmatchedContainerIds	A flag indicating if event files that cannot be matched to reagent cartridges in the LIMS should be archived after a certain period of time (true), or continually reprocessed (false).	false Note: To prevent the `gls_events` file directory from becoming cluttered, it is recommended that the value of this property is set to 'true'.
miseq.v6.seqservice.ignoreUnmatchedContainerIdsWaitDays	The number of days between when the event is created and the event file is archived.	14
miseq.v6.seqservice.synchronizationPeriod	Invocation period in seconds.	60

Support for Multiple seqservice.netPathPrefixSearch Property Values

It is possible to configure support for multiple, identical seqservice.netPathPrefixSearch property values. For details, refer to *Configure Multiple Identical netPathPrefixSearch Values* on page 2596.

Reagent Categories/Label Groups Installed

Reagent categories/ label groups are installed with the PCWP workflow configuration slices.

Reagent Kits Installed

The following reagent kits are included in the Illumina MiSeq Integration:

- MiSeq Reagent Kit

Control Types Installed

The following control types are included in the Illumina MiSeq Integration:

- PhiX v3

Container Types Installed

The following container types are included in the Illumina MiSeq Integration:

- MiSeq Reagent Cartridge

For more information, refer to *Support for Container Types* on page 2192.

Instrument Integration

To ensure that your Illumina instrument warranty remains valid, the instrument integration must be performed and maintained by the Clarity LIMS Support team. To perform this integration, the Support team will require remote access to the instrument while it is idle.

Below is an overview of the steps performed by the LIMS support team when configuring the instrument for use with the Illumina MiSeq Integration.

Configuring the Illumina Instrument

1. Create directory on the local computer to hold the batch files. These batch files write event files to the network attached storage (NAS) share(s).
2. Create directory on the NAS to hold the event files.
3. Modify Illumina software configuration files to call the batch files that create the event files.
4. Update sequencing service default database properties to match the specifics of the installation.

Support for Container Types

All one-dimensional container types with both numeric rows and numeric columns are supported.

Rules and Constraints

This integration operates with the following constraints:

- The reagent cartridge ID must be unique. There should not be multiple reagent cartridge containers in the system with the same name.
- The reagent cartridge ID must be scanned as the reagent cartridge Container Name on the Denature and Dilute (MiSeq v1.0) step.

Illumina MiSeq Integration v6.0 Validation and Troubleshooting

This guide explains how to validate the installation of the Illumina MiSeq Integration Package v6.0.

The validation process involves:

- Running samples through the Library Prep Validation v1.0 workflow. The workflow contains a single-step protocol that models the library prep required to produce normalized libraries. At the end of the step, the normalized libraries are automatically advanced to the MiSeq Sequencing v1.0 workflow.
- Running normalized libraries through the MiSeq Sequencing v1.0 workflow. This validates the following:
  - Automated generation of a sample sheet for use with the MiSeq Control Software (MCS).

- Automated tracking of the MiSeq sequencing run and parsing of run statistics into Clarity LIMS, including run status and metrics of sequencing run
- Sequencing run parameters
- Real Time Analysis v1 (RTA) run directory location and other run specific information

Validation Steps

Activate Workflows, Create Project, and Add and Assign Samples

The following steps set up Clarity LIMS in preparation for running samples through the Library Prep Validation v1.0 and MiSeq Sequencing v1.0 workflows.

1. Activate the Workflows

- In the LIMS configuration area, activate the Library Prep Validation v1.0 and MiSeq Sequencing v1.0 (shown below) workflows.

Figure 32 Workflow configuration and activation - LIMS v4.2

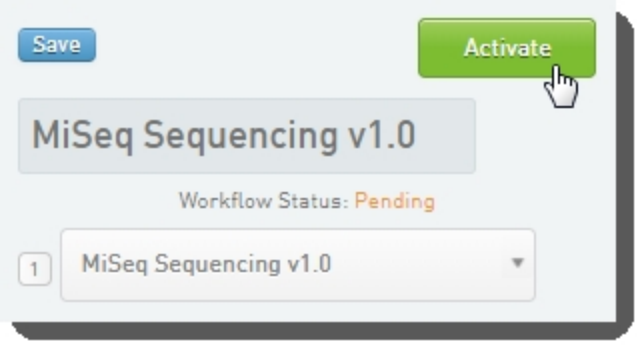
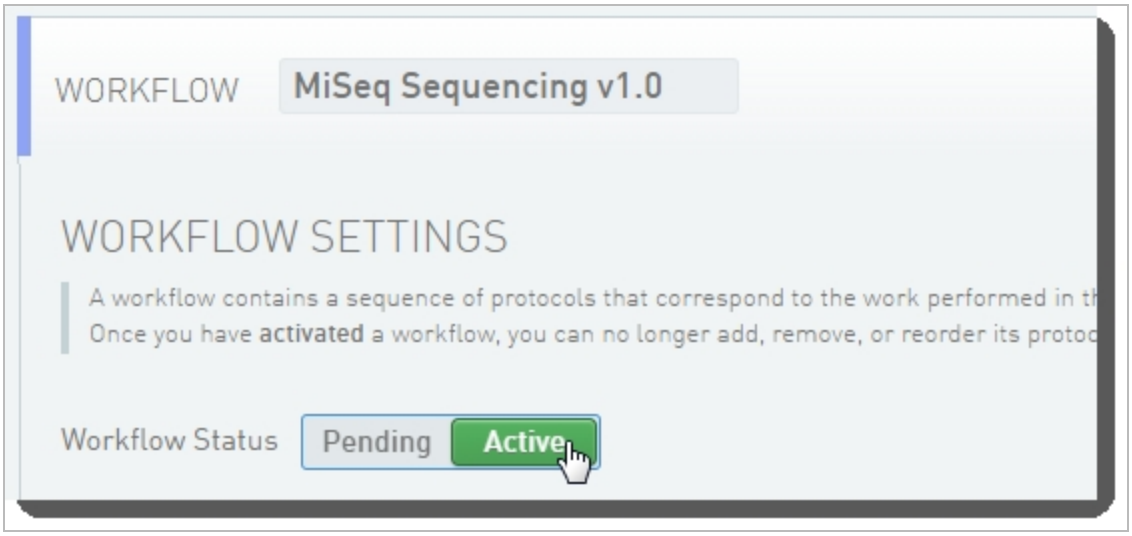
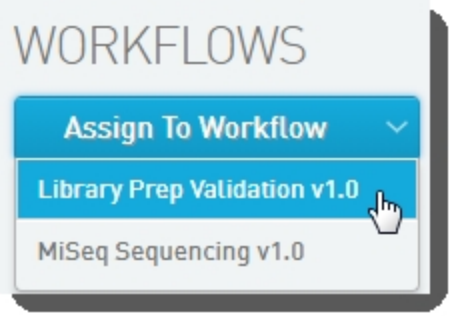


Figure 33 Workflow configuration and activation - LIMS v5.0



2. Create a Project, Add Samples

1. On the Projects and Samples screen, create a project and add samples to it.
2. Assign your samples to the Library Prep Validation v1.0 workflow.

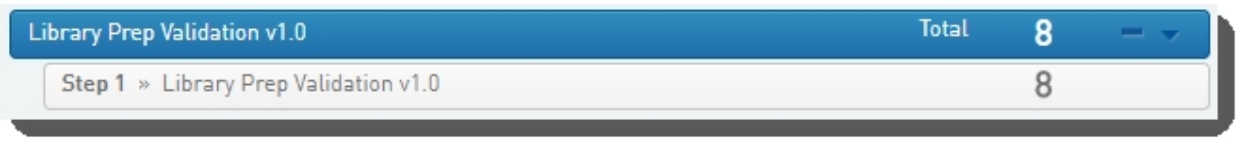


Library Prep Validation v1.0 Protocol

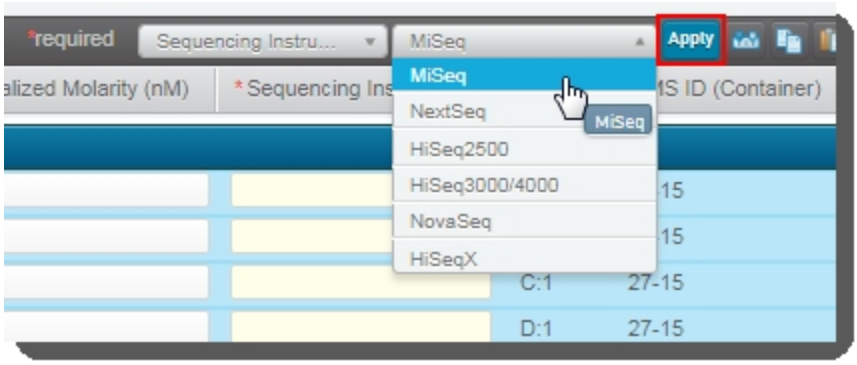
This single-step protocol models the library prep required to produce normalized libraries that are ready for the MiSeq Sequencing v1.0 workflow.

Run Library Prep Validation v1.0 Step

1. In Lab View, locate the Library Prep Validation v1.0 protocol. You will see your samples queued for the Library Prep Validation v1.0 step.



2. Add the samples to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Placement screen, in the Placed Samples area on the right, in the container field, scan or enter the barcode of the destination container.
    - Select the samples from the Samples to be Placed area on the left, and then drag them over to the container wells on the right.
    - Select Add Labels.
5. On the Add Labels screen, select labels on the left, and drag them over to the container on the right to assign them to placed samples.
    - Select Record Details.
6. On the Record Details screen, select all of the samples in the Sample Details table and then in the Sequencing Instrument dropdown list, select MiSeq.

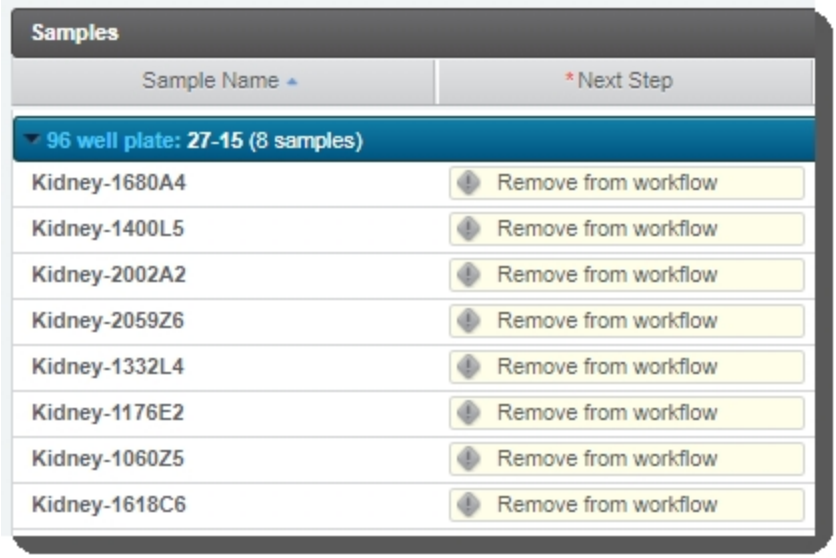


- Select Apply to set this value for all selected samples.

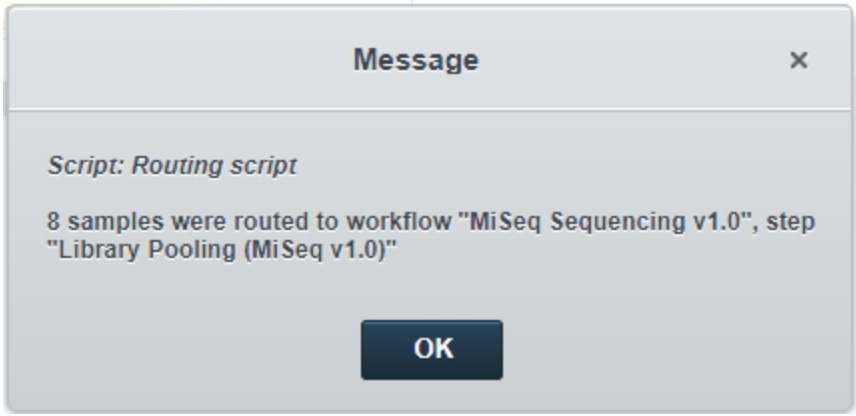


- Select Next Steps.

7. On the Assign Next Steps screen, the next step is already set to Remove from Workflow (this is required by the Routing script automation).



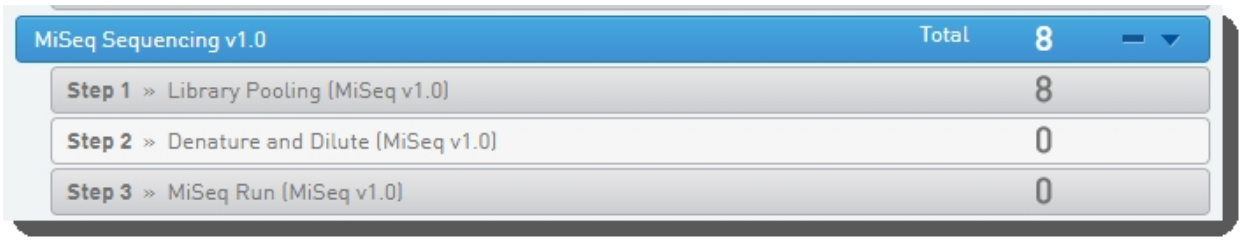
8. Select Finish Step.The Routing script automation is triggered and samples are automatically routed to the first step in the MiSeq Sequencing v1.0 protocol - Library Pooling (MiSeq v1.0).



MiSeq Sequencing v1.0 Protocol

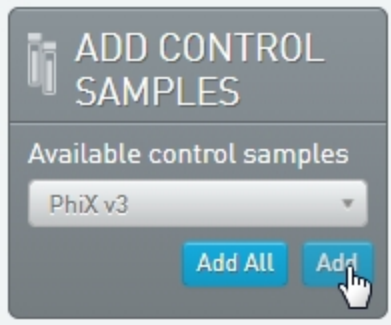
1. Run Library Pooling (MiSeq v1.0) Step

1. In Lab View, locate the MiSeq Sequencing v1.0 protocol. You will see your samples queued for the Library Pooling (MiSeq v1.0) step. Click the step to proceed to the Queue screen.

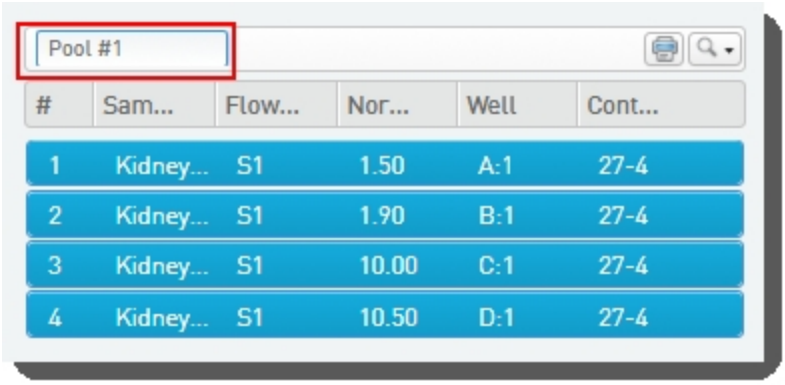


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2. On the Queue screen, add the samples to the Ice Bucket. In the Add Control Samples panel, add the PhiX v3 control sample to the Ice Bucket.



3. Select View Ice Bucket.
4. On the Ice Bucket screen, select Begin Work.
5. On the Pool Samples screen: Create a pool of samples by dragging samples into the Pool Creator.
    - Name the pool, or accept the default name - Pool #1.

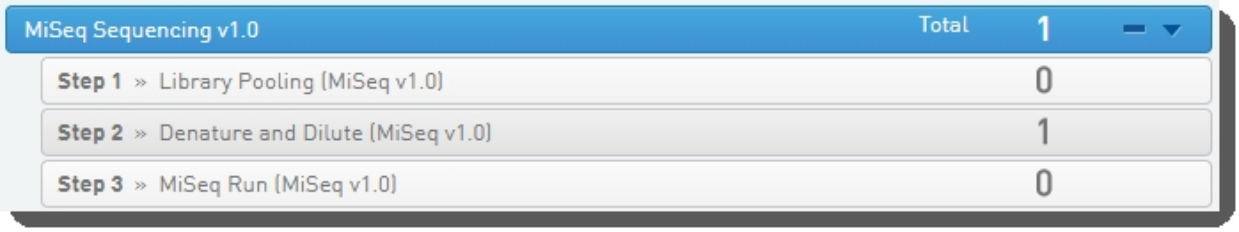


    - Select Place Samples.
6. On the Placement screen, select the pool in the Samples to be Placed area on the left and drag it over to the container on the right.
    - Select Record Details.
7. On the Record Details screen, select Next Steps.
8. On the Assign Next Steps screen, next step is already set to Denature and Dilute (MiSeq v1.0).
9. Select Finish Step.

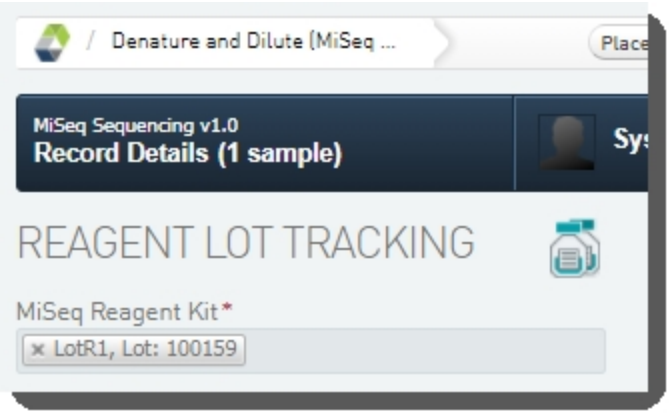
2. Run Denature and Dilute (MiSeq v1.0) Step

1. In Lab View, locate the MiSeq Sequencing v1.0 protocol. You will see your pooled samples queued for the Denature and Dilute (MiSeq v1.0) step. Select the step to proceed to the Queuescreen.

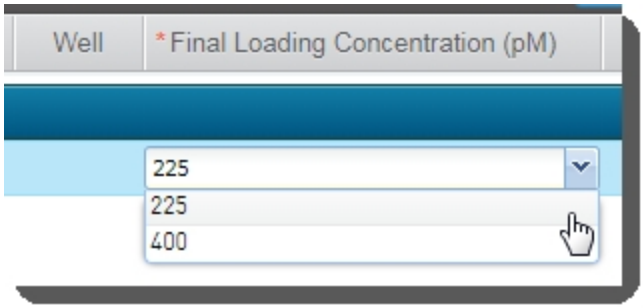
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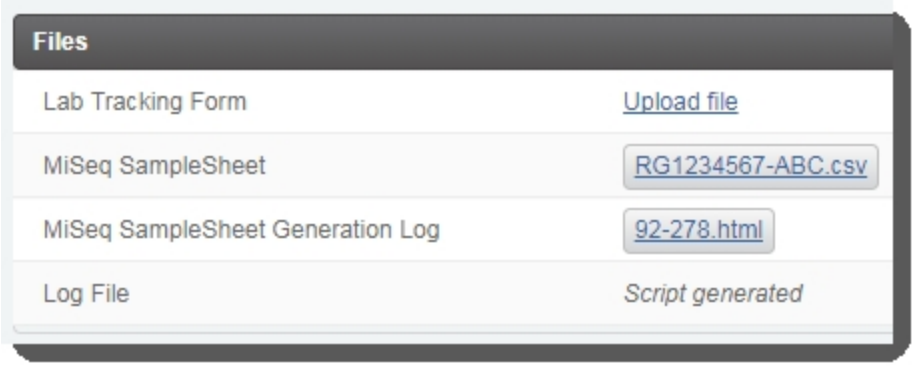
2. On the Queue screen, add the pool to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Placement screen, scan the MiSeq reagent cartridge barcode into the MiSeq Reagent Cartridge field.
    - Place the pool of samples into the reagent cartridge.
    - Select Record Details.
5. On the Record Details screen, under Reagent Lot Tracking, select the reagent lot used in the step. You may need to add/activate the lot on the Reagents and Controls screen.



    - Populate the fields in the Step Details table, as appropriate. Note that Workflow and Read 1 Cycles are required fields.
    - In the Sample Details table, enter the Final Loading Concentration. You may select from the two preset options: 225 (for PCR-free workflows) or 400 (for Nano workflows), or enter a different value.



- Select Generate MiSeq SampleSheet.
- Clarity LIMS generates the samplesheet and attaches it and a log file to placeholders in the Files area of the Record Details screen. Download the files and validate their format and content.



- Select Next Steps.

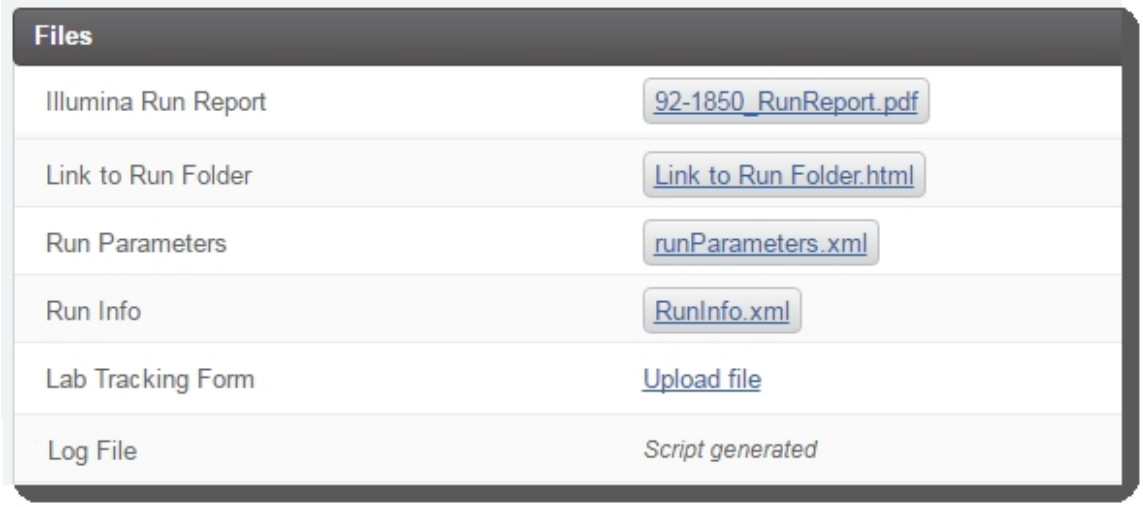
6. On the Assign Next Steps screen, samples are already assigned to the MiSeq Run (MiSeq v1.0) step.
7. Select Finish Step.

3. Run MiSeq Run (MiSeq v1.0) Step

1. In Lab View, locate the MiSeq Sequencing v1.0 protocol. You will see your pool of samples queued for the MiSeq Run (MiSeq v1.0) step. Select the step to proceed to the Queue screen.
2. Add the pool to the Ice Bucket and select View Ice Bucket.
3. On the Ice Bucket screen, select Begin Work.
4. On the Record Details screen, the fields in the table are read-only. Once the run completes, the integration will automatically: Populate the fields.
    - Attach files to the Illumina Run Report, Link to Run Folder, Run Parameters, and Run Info placeholders. (Note that the Log File is attached once the next step for samples has been assigned by the Next Step - Advance automation.)

    Do not continue to step 5 and complete the step until the Illumina Run Report has been attached.

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- Populate the fields in the Sample Details table. For details, refer to *Illumina MiSeq Integration v6.0 Configuration and User Interaction* on page 2163.

5. Select Next Steps.
6. On the Assign Next Steps screen, the next step is already set to Mark protocol as complete.
7. Select Finish Step.

Validating Creation of Event Files

This validation checks the following information:

- The DESTINATION_PATH is correctly configured.
- The instrument computer can access and write to the DESTINATION_PATH.
- There are no syntax errors in the Clarity LIMS batch file.

Use the following steps to confirm that event files are created by the batch file in the destination path. The steps assume that the default configuration has been successfully imported.

1. In `C:\gls`, double-click the batch file (`gls_event_mcs_rta.bat` or `gls_event_mcs_rta_lrm.bat`, depending on MiSeq version).
2. Confirm that an empty event file appears in the configured DESTINATION_PATH.

Manual execution of the batch file produces an output resembling the following example:

```
Filename:
event-EndRun-07295667.txt
Contents:
cycleNumber =
runFolder =
netFolder =
readType =
```


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```
eventType = EndRun
softwareType = MCS
```

Troubleshooting

If an automation trigger does not appear to run the corresponding scripts, refer to the following articles in the Clarity LIMSAPI & Database documentation:

- *Troubleshooting Automated Informatics*
- *Troubleshooting EPP / Automation*

If an error occurs that does not provide direction on how to proceed, confirm the version of the installed MiSeq Integration Package. To confirm the version, run the following command from the server console:

```
rpm -qa | grep -i miseq
```

If the error is related to data parsing, retrieving run results data, or report values not appearing as expected, review the `MiseqIntegrator.log` file. The file is located at `/opt/gls/clarity/extensions/Illumina_MiSeq/v6/SequencingService`.

Additional troubleshooting information for this integration is provided in the *Illumina Instrument Integrations FAQ* on page 2598.

If you are unable to resolve the issue, contact the Clarity LIMS Support team.

Illumina MiSeq Integration 6.0 Release Notes

Overview: Workflow and Instrument Integrations for Illumina MiSeq 6.0

- Updated configurable SampleSheet generation SampleSheet generation is now done using the updated Template File Generator tool, which enables updates of the provided template for SampleSheet generation in order to add additional headers and columns.
  - Template provided with accompanying configuration generates the MiSeq SampleSheet.
  - When a new custom template is created, it can be uploaded using the Template File Management feature introduced in BaseSpace Clarity LIMS 5.1.
- New delivery mechanism for MiSeq Integration workflow configuration MiSeq Workflow configuration now delivered using a robust, Preconfigured Workflow Package installer.
  - Install history is recorded with version and install date information for better tracking and troubleshooting of installation.
- Simplified Library Prep step for validationSingle step mock library prep protocol included with configuration to facilitate quick validation of installed MiSeq 6.0 Sequencing Protocol.
- Supports MiSeq with Local Run ManagerSupport added in this release, to work with Local Run Manager (LRM) connected Miseq Control Software.

Bugs resolved in this release:

- Updated the Sequencing Interops Metrics parser to record more accurate metrics using the same parsing libraries as the instrument and SAV.

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- Resolved a bug that was causing the Run Reports not attaching to MiSeq integration.
- Fixes of various internal automated test issues including smoketests.

Illumina MiSeq Instrument Interface

Note:
To ensure that your Illumina instrument warranty remains valid, this integration must be performed and maintained by the BaseSpace Clarity LIMS Support team as needed.

Overview of the BaseSpace Clarity LIMS ↔ Illumina MiSeq Instrument Interface

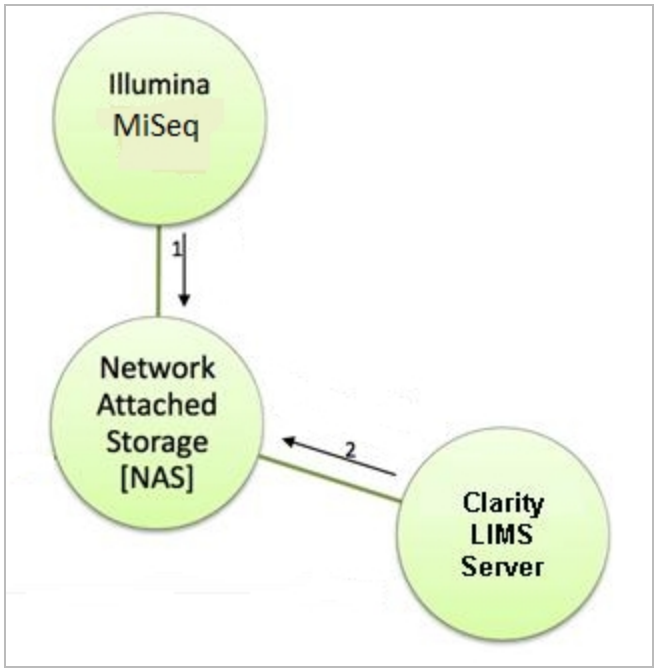


Image Action #	Description
0	MiSeq Run (MiSeq) 4.0 initiated in the LIMS.
1	1. End Run event fires on Illumina instrument. 2. Instrument fires configured LIMS script. 3. Script writes End Run event to Network Attached Storage (NAS) root folder in \gls_events .

Image Action #	Description
2	1. Timer-based daemon on the LIMS server wakes up and looks for events. 2. Daemon discovers End Run event and processes it. 3. Daemon uses the supplied process run directory to locate the run folder on the NAS. 4. Daemon parses key information from the run folder, including the Reagent Cartridge ID. 5. Daemon uses the LIMS REST API to do the following: • Populate process UDFs: Finish Date, Run Type, Status, Flowcell ID, Flowcell Version, Experiment Name, Read 1 Cycles, Index 1 Read Cycles, Index 2 Read Cycles, Read 2 Cycles, Output Folder, Run ID, Reagent Cartridge ID, Reagent Cartridge Part #, PR2 Bottle ID, Chemistry, Workflow • Examine list of files to collect, and attach any that are available: – RunInfo.xml, runParameters.xml, Link to Run Folder.html • Run statistics are parsed from several binary InterOp files and stored (visible in the MiSeq run). • Illumina Run Report invoked, generated, and attached.

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MiSeqDx Integrations

The Illumina MiSeqDx instrument integration provides preconfigured workflows that map to established lab protocols and steps used with the MiSeqDx instrument. The integration also supports the associated library prep kits, reagent kits, and assays used in these protocols.

MiSeqDx Integration v1.10 Configuration and User Interaction

The Illumina MiSeqDx Integration Package v1.10 supports the integration between Clarity LIMS and MiSeqDx instruments. The integration allows for automated tracking of an Illumina sequencing run in Clarity LIMS and includes the following functionality:

- Tracking instrument run status.
- Generating a run report.
- Capturing and parsing run statistics.
- Uploading variant call files to the LIMS.

In addition, this integration provides automated generation of a sample sheet file for use with the MiSeqDx Operating Software (MOS).

This documentation describes the integration between Clarity LIMS and the MiSeqDx system. It includes information about protocols and automations, configuration options, installed components, and rules and constraints.

Integration Features

- MiSeqDx RPM that supports Diagnostic (Dx) mode.
- MiSeq RPM that supports Research (RUO) mode.
- Preconfigured Clarity LIMS protocols that map to library prep and sequencing lab protocols and instrument runs.
- Preconfigured protocols that allow for validation of the sample sheet generation and index placement functionality.
- Automatic generation of:
  - A sample sheet file for use with the MOS (Diagnostic mode) instrument software.
  - A run report, which includes the current run statistics.
- Automated tracking of the following information in Clarity LIMS:
  - Progress and metrics of a MiSeqDx sequencing run
  - Per-instrument sequencing runs

  - Sequencing run parameters
  - Real-Time Analysis (RTA) run directory location and other run specific information
- The ability to monitor the run status (cycle number) across multiple instruments from within Clarity LIMS.
- The ability to attach Variant Call Format (VCF) files to Clarity LIMS.

Protocols

The following protocols are included in MiSeqDx Integration Package v1.10:

- CF 139-Variant Assay Library Prep 1.2
- CF Clinical Sequencing Assay Library Prep 1.2
- Illumina SBS MiSeqDx (CF 139-Variant Assay) 1.2
- Illumina SBS MiSeqDx (CF Clinical Sequencing Assay) 1.2
- Illumina SBS MiSeqDx (Universal Kit) 1.2
- Universal Kit Library Prep 1.2

There are three validation protocols. Each protocol is included in a workflow with the same name. The protocols are as follows.

- MiSeqDx Validation (CF 139-Variant Assay) 1.2
- MiSeqDx Validation (CF Clinical Sequencing Assay) 1.2
- MiSeqDx Validation (Universal Kit) 1.2

In Assay workflows, include a QC validation protocol between the Library Prep and Illumina SBS protocols. The default workflow does not include a QC protocol.

Each validation protocol includes the following steps from the Library Prep and Illumina SBS MiSeqDx protocols:

- Extension-Ligation of Bound Oligos (Library Prep step)
- PCR Amplification (Library Prep step)
- Library Pooling (MiSeqDx) (Illumina SBS MiSeqDx step)
- Denature, Dilute and Load Sample (Illumina SBS MiSeqDx step)
- MiSeqDx Run (MiSeqDx) (Illumina SBS MiSeqDx step)
- Variant Calling (MiSeqDx) (Illumina SBS MiSeqDx step)

For instructions on using the MiSeqDx Validation (CF 139-Variant Assay) 1.2 protocol to validate automated sample sheet generation, refer to *MiSeqDx Integration v1.10 Validation and Troubleshooting* on page 2229.

Automations

The following table lists the automations included in this integration, and the steps on which they are configured.

Table 471 MiSeqDx Integration Default Automations

Protocol	Steps & Automations
• CF 139-Variant Assay Library Prep 1.2 • Universal Kit Library Prep 1.2	On PCR Amplification (CF 139-Variant Assay / Universal Kit) 1.2 step: • Auto Place Indexes • Validate Index Placement
• Illumina SBS MiSeqDx (CF 139-Variant Assay) 1.2 • Illumina SBS MiSeqDx (CF Clinical Sequencing Assay) 1.2 • Illumina SBS MiSeqDx (Universal Kit) 1.2	On Library Normalization (MiSeqDx) 1.2 step: • Create Normalization CSV
• Illumina SBS MiSeqDx (CF 139-Variant Assay) 1.2 • Illumina SBS MiSeqDx (CF Clinical Sequencing Assay) 1.2 • Illumina SBS MiSeqDx (Universal Kit) 1.2 • MiSeqDx Validation (CF 139-Variant Assay) 1.2 • MiSeqDx Validation (CF Clinical Sequencing Assay) 1.2 • MiSeqDx Validation (Universal Kit) 1.2	On Denature, Dilute and Load Sample (CF 139-Variant Assay / CF Clinical Sequencing Assay / Universal Kit) 1.2 step: • Generate MiSeqDx Sample Sheet • Validate Container Name • Validate Single Input
• Illumina SBS MiSeqDx (CF 139-Variant Assay) 1.2 • Illumina SBS MiSeqDx (CF Clinical Sequencing Assay) 1.2 • Illumina SBS MiSeqDx (Universal Kit) 1.2 • MiSeqDx Validation (CF 139-Variant Assay) 1.2 • MiSeqDx Validation (CF Clinical Sequencing Assay) 1.2 • MiSeqDx Validation (Universal Kit) 1.2	On MiSeqDx Run (MiSeqDx) 1.2 step: • AUTOMATED - Run Report Generation • Verify Report Status

Library Prep Protocols

This section discusses the Library Prep protocols, steps, and automations included in the MiSeqDx v1.10 Integration Package.

Index Placement and Validation Automations

This section discusses the index placement and validation automations configured on the PCR Amplification 1.2 Library Prep steps.

The example workflow uses the CF 139-Variant Assay Library Prep 1.2 protocol. However, important considerations related to the Universal Kit Library Prep 1.2 and CF Clinical Sequencing Assay Library Prep 1.2 protocols are noted.

By default, in the CF 139-Variant Assay Library Prep 1.2 and the Universal Kit Library Prep 1.2 protocols, the PCR Amplification 1.2 step includes two automations. Both automations invoke the `place_indexes` script with different options.

- Auto Place Indexes—Automatically invoked on entry to the Add Reagents screen.
- Validate Index Placement—Automatically invoked on exit from the Add Reagents screen.

A list of reagents installed with this configuration is provided in *Installed Components* on page 2223.

Good laboratory practices mandate that a positive control DNA sample and a negative (no-template) control sample are included in every run. The positive control DNA sample should be a well-characterized sample with a known CFTR mutation.

How it Works

On entry to the Add Reagents screen

The Auto Place Indexes automation is invoked. This in turn invokes the `place_indexes` script, which places reagent indexes into the container based on the pattern defined in a specified index placement pattern file. (The Step Automation Details table that follows shows the pattern file names and their location.)

Reagent indexes are assigned to each sample in the destination container according to the specified index placement pattern file. Note the following:

- The automatic index placement pattern for Universal Kit can be modified.
- The automatic index placement pattern for CF 139-Variant Assay should not be modified.
- If an 8-sample reagent category / label group is selected (refer to step 1 of *User Interaction* on page 2209), index placement must be performed manually.

  Index placement must be performed manually for the CF Clinical Sequencing Assay Library Prep 1.2 protocol, which only uses 8-sample reagent categories / label groups.

- Upon completion of the reagent index placement, a success message displays. Select OK to close the message. Indexes with the pattern specified in the placement file are then assigned to the samples.

On exit from the Add Reagents screen

The Validate Index Placement automation invokes the `place_indexes` script once again, this time to validate the index placement:

- If the index placement is user-modifiable (Universal Kit only), any changes made by the user are preserved.
- If the index placement cannot be modified (CF 139-Variant Assay), any changes made by the user are disregarded and the automatic index placement is restored.

Step Automation Details

The following table shows the automation commands configured on the Auto Place Indexes and Validate Index Placement automations during the PCR Amplification (CF 139-Variant Assay) 1.2 step.

Table 472 PCR Amplification 1.2 Step Automation Details

Automation	Trigger	Command
PCR Amplification (CF 139-Variant Assay) 1.2 step:		
Auto Place Indexes	On entry to Add Reagents screen	`bash -l -c "/opt/gls/clarity/bin/java -cp /opt/gls/clarity/extensions/ngs-common/v5/EPP/PlacementHelper.jar place_indexes -i {stepURI:v2} -u {username} -p {password} -f /opt/gls/clarity/extensions/conf/miseqdx/placementpatterns/placement_pattern_index_cf139_01.tsv -ic 'CF 139-Variant Assay 8-Sample Indexes' -locked true"`
Validate Index Placement	On exit from Add Reagents screen	`bash -l -c "/opt/gls/clarity/bin/java -cp /opt/gls/clarity/extensions/ngs-common/v5/EPP/PlacementHelper.jar place_indexes -i {stepURI:v2} -u {username} -p {password} -f /opt/gls/clarity/extensions/conf/miseqdx/placementpatterns/placement_pattern_index_cf139_01.tsv -ic 'CF 139-Variant Assay 8-Sample Indexes' -locked true -fc true"`
PCR Amplification (Universal Kit) 1.2 step:		

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Automation	Trigger	Command
Auto Place Indexes	On entry to Add Reagents screen	`bash -l -c "/opt/gls/clarity/bin/java -cp /opt/gls/clarity/extensions/ngs-common/v5/EPP/PlacementHelper.jar place_indexes -i {stepURI:v2} -u {username} -p {password} -f /opt/gls/clarity/extensions/conf/miseqdx/placementpatterns/placement_pattern_index_universal_01.tsv -ic 'Universal Kit 8-Sample Indexes'"`
Validate Index Placement	On exit from Add Reagents screen	`bash -l -c "/opt/gls/clarity/bin/java -cp /opt/gls/clarity/extensions/ngs-common/v5/EPP/PlacementHelper.jar place_indexes -i {stepURI:v2} -u {username} -p {password} -f /opt/gls/clarity/extensions/conf/miseqdx/placementpatterns/placement_pattern_index_universal_01.tsv -ic 'Universal Kit 8-Sample Indexes' -fc true"`

User Interaction

The section describes the user interaction involved in running samples through the PCR Amplification (CF 139-Variant Assay) 1.2 step.

1. Run the PCR Amplification (CF 139-Variant Assay) 1.2 step. Add samples to the Ice Bucket and select Reagent Label Options.
2. Select one of the following options:
   - For manual placement of indexes with less than eight samples, select CF 139-Variant Assay 8-Sample Indexes.
   - For automatic placement of indexes with more than eight samples, select CF 139-Variant Assay Indexes.
3. Select Begin Work.
4. On the Record Details screen, select Add Reagents.
5. A message box displays, notifying you that a custom script is in progress. Upon script completion, a success message displays. Select OK to proceed.
6. Make sure the indexes with the pattern specified in the placement file are assigned to samples and displayed.

If you are running the Universal Kit protocol, you may modify the index placement. If you are running the CF 139-Variant Assay protocol, index placement is not modifiable. If you attempt to modify the placement pattern, your changes will be reverted on exit from the Add Reagents screen and the automatic placement will be restored.

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7. Select Next Steps.
   Clarity LIMS does the following:
   - Validates the index placement.
   - Reverts any changes made to index placement in the CF 139-Variant Assay protocol.
8. A success message displays. Select OK to proceed.

Step 1: Hybridization of Oligo Pool (CF 139-Variant Assay) 1.2

The following process is used to hybridize the oligo pool.

1. Add samples to Ice Bucket.
2. Add Negative Control and Positive Control samples to Ice Bucket.

   Good laboratory practices mandate that a positive control DNA sample and a negative (no-template) control sample are included in every run. The positive control DNA sample should be a well-characterized sample with a known CFTR mutation.
3. Select Begin Work.
4. On the Placement screen, select all samples and place in container. Select Record Details.
5. On the Record Details screen, under Reagent Lot Tracking, set the following values:
   - gDNA Concentration (ng/uL): 50 ng/ul
   - gDNA Volume (uL): 5 uL
   - Select the reagent lots for CF 139-Variant Assay-Oligo Pool and Hybridization Buffer.
6. Select Next Steps.
7. Select Removal of Unbound Oligos (CF 139-Variant Assay) 1.2 as the next step.
8. Select Apply.
9. Select Finish Step.

Step 2: Removal of Unbound Oligos (CF 139-Variant Assay) 1.2

The process below is used to remove unbound oligos.

1. Add samples to Ice Bucket.
2. Select Begin Work.
3. On the Placement screen, select all samples and place in container.
4. Select Record Details.
5. On the Record Details screen, under Reagent Lot Tracking, select the reagent lots for Stringent Wash Buffer and Universal Wash Buffer.
6. Select Next Steps.
7. Select Extension-Ligation of Bound Oligos (CF 139-Variant Assay) 1.2 as the next step.
8. Select Apply.

9. Select Finish Step.

Step 3: Extension-Ligation of Bound Oligos (CF 139-Variant Assay) 1.2

The process below is used to add reagents to the Extension-Ligation Mix field.

1. Add samples to Ice Bucket. Select Begin Work.
2. On the Placement screen, select all samples and place in container.
3. Select Record Details.
4. On the Record Details screen, under Reagent Lot Tracking, select the reagent lots for Extension-Ligation Mix.
5. Select Next Steps.
6. Select PCR Amplification (CF 139-Variant Assay) 1.2 as the next step.
7. Select Apply.
8. Select Finish Step.

Step 4: PCR Amplification (CF 139-Variant Assay) 1.2

1. Add samples to Ice Bucket.
2. Select one of the following Reagent Label Options:
    - If you are working with eight samples or less, select CF 139-Variant Assay 8-Sample Indexes.
    - If you are working with more than 8 samples, select CF 139-Variant Assay Indexes.
3. Select Begin Work.
4. On the Placement screen, select all samples and place in container. Select Add Reagents.
   On entry to the Add Reagents screen, the Auto Place Indexes automation is invoked and the reagents are placed on the samples.
5. Once placement is complete, a message displays indicating that the index pattern has been applied successfully. Select OK.
6. On Add Reagents screen, reagents are already placed. Select Record Details.
7. On exit from the Add Reagents screen, the index placement is validated. Once validation is complete, a message displays indicating that the index pattern has been validated on all samples successfully. Select OK.
8. On the Record Details screen, under Reagent Lot Tracking, enter the lots for the following fields:
    - Index Primers
    - NaOH
    - PCR Master Mix
    - PCR Polymerase

9. Select Next Steps.
10. Select PCR Clean-Up (CF 139-Variant Assay) 1.2 as the next step.
11. Select Apply.
12. Select Finish Step.

Step 5: PCR Clean-Up (CF 139-Variant Assay) 1.2

1. Add samples to Ice Bucket.
2. Select Begin Work.
3. On the Placement screen, select all samples and place in container. Select Record Details.
4. On the Record Details screen, under Reagent Lot Tracking, select the reagent lots for the following:
   - Elution Buffer
   - EtOH
   - PCR Clean-up Beads
5. Select Next Steps.
6. Select Mark protocol as complete as the next step. Select Apply.
7. Select Finish Step.

Illumina SBS MiSeqDx v1.2 Protocols

The following sections describe the key features of the Illumina SBS MiSeqDx v1.2 protocols. These sections also describe the user interaction involved at these points in the workflow.

- Library normalization CSV file generation
- Reagent cartridge name validation
- Sample sheet generation
- Primary analysis (sequencing) results parsing, which includes generation of the run report
- Secondary analysis results parsing

Library Normalization File Generation

In each of the Illumina SBS MiSeqDx protocols, the Library Normalization (MiSeqDx) v1.2 step includes automated calculation of normalization buffer volumes. The results are provided in an autogenerated comma-separated file that is attached to the step.

How it Works

Buffer volumes are calculated by the normalizationBufferVolumes script. The script is invoked by the Create Normalization CSV automation when you select a button on the Record Details screen.

For details on the normalizationBufferVolumes script, refer to *Normalization Buffer Volumes* on page 2577.

User Interaction

1. On the Record Details screen of the Library Normalization (MiSeqDx) v1.2 step, enter values in for the following items:
    - Library volume (µl) transferred to destination plate
    - Normalized conc (nM)
    - Maximum destination container volume (µl)
    - Any other required fields
2. Select Create Normalization CSV to start the automation.
   When the normalizationBufferVolumes script has completed, the Normalization buffer volumes CSV file is generated and attached to the step. This file is in the Files area of the Record Details screen.

Reagent Cartridge Name Validation

In each of the Illumina SBS MiSeqDx and Validation protocols, the Denature, Dilute and Load Sample 1.2 step includes automated validation of the MiSeqDx reagent cartridge name.

How it Works

Automatically initiated on exit from the Placement screen of the step, the Validate Container Name automation validates the reagent cartridge name to ensure:

- The reagent cartridge Container Name has been renamed and is not the same as its Clarity LIMS ID.

```
if (output.container.name == output.container.node.@limsid) { fail(::The
Container Name cannot be the same as its LIMS ID. Please scan the Reagent
Cartridge RFID.::) }
```

- The Container Name contains only letters and numbers, does not have special characters, and is in the format `AB#######-XYZ` (eg, `RG1234567-ABC`).

```
else if (!output.container.name.matches(::^[a-zA-Z0-9-]*$::)) { fail(::The
Container Name can contain letters, numbers and dashes only. No other special
characters are allowed. Please scan the Reagent Cartridge RFID.::) };
```

- Sets the Reagent Cartridge Barcode field attached to the output that is from the sample input.

```
output.::Reagent Cartridge Barcode:: = output.container.name;
```

- Pads the suffix (the characters after the dash) of the Container Name so that it is at least five characters long. The Container Name is used by the MiSeqDx Sequencing Service to match the run data to the Clarity LIMS step in the MiSeqDx Run (MiSeqDx) step.

```
def match = output.container.name =~ /([a-zA-Z0-9]+-)([a-zA-Z0-9]{0,5})$/;
if (match.matches()) {
```


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```
int barcodeSuffixLength = 5;
int zerosToAdd = barcodeSuffixLength - match.group(2).size();
output.container.::name:: = match.group(1).toString() + ::0::*zerosToAdd +
match.group(2).toString()
}
```

User Interaction

1. On the Placement screen of the Denature, Dilute and Load Sample 1.2 step, set up the reagent cartridge as follows.
   a. Using an RFID scanner, scan the RFID of the MiSeqDx reagent cartridge into the Container Name field.
   b. Place the pool of samples into the reagent cartridge.
2. Select Record Details.

On exit from the Placement screen, the automation is automatically triggered and validates the Container Name.

Sample Sheet Generation

In each Illumina SBS MiSeqDx and Validation protocol, automated sample sheet generation is configured on the Record Details screen of the Denature, Dilute and Load Sample 1.2 step. This is when the samples are loaded onto the container that will be placed in the instrument.

The integration allows for generation of a sample sheet designed to be used with the MOS (Diagnostic mode) instrument software.

By default, one MiSeqDx instrument sample sheet is created for the reagent cartridge loaded during the step. The placeholders and sample sheet files in the following table are generated by the Denature, Dilute and Load Sample 1.2 step.

Generated Placeholders and Sample Sheet Files

Item	Description
Lab Tracking Form	This item allows you to manually attach a lab-specific tracking form to the step.
MiSeqDx Sample Sheet file	• This CSV file is automatically generated by the LIMS for use with the MOS or MCS software. • May be opened as a text file, or as an MS Excel spreadsheet.

Item	Description
MiSeqDx Sample Sheet Generation Log	Automatically generated by Clarity LIMS, this log file captures any errors encountered when generating the MiSeqDx sample sheet.

How it Works

The sample sheet generation automation is configured on the Record Details screen of the Denature, Dilute and Load Sample 1.2 step.

- This automation is triggered by a button on the Record Details screen.
- The automation sets the value of the Progress field on the sample to 'Library ready for sequencing'.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -u {username} -p {password} -i {processURI:v2}
script:setUDF -f 'Progress' -t '//input/@uri->//sample/@uri' -v 'Library ready
for sequencing'
```

- The automation starts a script that generates the sample sheet.

```
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/miseqdx/miseqdx-
extensions.jar -u {username} -p {password} -i {processURI:v2} script:generate_
miseqdx_sample_sheet -c {compoundOutputFileLuid1} -e {compoundOutputFileLuid2} -
-useSampleLimsID true --isMiSeqDx true
```

> To maintain unique values in the Sample_Name column of the sample sheet, the sample sheet generation automation script has the `--useSampleLimsID` parameter set to true. The `--appendLimsID` parameter must not be set to true.
>
> For the automation to display the correct instrument name when it is completed, the `--isMiSeqDx` parameter must be set to true.

- The automation labels the derived samples that do not have reagent labels. The label value applied is NoIndex.

```
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -u {username} -p {password} -i {processURI:v2}
script: labelNonLabeledOutputs -l 'NoIndex'
```

User Interaction

For more information, refer to *MiSeqDx Integration v1.10 Validation and Troubleshooting* on page 2229.

Supporting Documentation

For more information on sample sheet generation, including details on file format and contents, master step and global fields, script parameters, and configuration options, refer to the following documentation:

- *Illumina MiSeqDx 1.x Sample Sheet Generation*—For details on the Diagnostic mode sample sheet designed for use with MOS software.
- *Sample Sheet Generation* on page 2214—For details on the Research mode sample sheet designed for use with MCS software.

Primary Analysis (Sequencing) Results Parsing

The MiSeqDx Run (MiSeqDx) step records information for each lane of the flow cell and generates a report summarizing the results. In addition, run parameters, run info, and a link to the run folder are automatically captured.

Generated and Captured Files

The following table describes the run information files, reports, placeholders, and links that Clarity LIMS automatically generates or captures during a sequencing run.

Table 473 Run Information Generated or Captured by MiSeqDx Run (MiSeqDx) v1.2 Step

Item	Description
Run Info Run Parameters	These XML files are automatically captured by Clarity LIMS from the instrument run folder. They include the key run parameters, many of which are parsed out into key master step and global fields.
Link to Run Folder	Automatically generated by Clarity LIMS, this is a link to the network run folder where the data that was captured from the instrument during the run is stored.

Item	Description
Illumina Run Report	• Automatically generated by Clarity LIMS, this report provides key information about the run and the samples on the flow cell. • Information includes the flow cell ID, run directory location, instrument name, as well as primary analysis metrics for the instrument – summarized per flow cell lane for the entire run, and individual reads in the case of paired-end runs. • These metrics are compared against the instrument's per lane averages, calculated using metrics from the last five sequencing runs. Any values outside of one standard deviation are highlighted. • The run report calculations assume that the flow cell has 8 lanes. If you are using a two-lane rapid-run flow cell, the calculated values will be incorrect.
Lab Tracking Form	This item allows you to manually attach a lab-specific tracking form to the step.

Metadata

The following metadata are stored as custom fields tagged to the MiSeqDx Run (MiSeqDx step). These fields are all read-only.

- Finish Date* – run completion date
- Run Type – Diagnostic or Research mode
- Status – current status of the sequencing run on the instrument
- Flow Cell ID
- Flow Cell Version
- Experiment Name – entered in software
- Read 1 Cycles
- Index 1 Read Cycles – intended Index cycles
- Index 2 Read Cycles – intended Index cycles
- Read 2 Cycles
- Output Folder – run folder root

- Run ID – the unique run ID
- Reagent Cartridge ID
- Reagent Cartridge Part #
- PR2 Bottle ID
- Chemistry
- Workflow

There are two additional master step fields in this step:

- Comments—multiline text field used for any comments that are attached to the run
- Report Status—hidden read-only field that tracks if the run report has been successfully uploaded

Date Run is populated with the date that the Begin Run event file is first picked up and associated with a step in Clarity LIMS, not the date of the Begin Run event itself (the date the run was performed on the instrument). This property is provided by default in Clarity LIMS and is hidden. It is used in the AUTOMATED - Run Report Generation automation.

Finish Date is populated as follows.

- If the End Run event contains a date in the format YYYY-MM-DD, Finish Date will be set to the date in the event file.
- If the End Run event does not contain a date or the date is in the wrong format, Finish Date will be set to the date when the event file is processed.

Primary Analysis Metrics

The following table lists the Real-Time Analysis (RTA) primary analysis metrics Clarity LIMS automatically captures and records, per read, for samples in each flow cell lane. These metrics are captured upon run completion.

Table 474 RTA Primary Analysis Metrics Captured by MiSeqDx Run (MiSeqDx) Step

RTA Primary Analysis Metrics	Corresponding Fields
Raw Yield (Gb)	Yield PF (Gb) R1 Yield PF (Gb) R2
% Bases >Q30	% Bases >=Q30 R1 % Bases >=Q30 R2
Cluster Density (K/mm^2)	Cluster Density (K/mm^2) R1 Cluster Density (K/mm^2) R2

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RTA Primary Analysis Metrics	Corresponding Fields
Clusters Raw	Clusters Raw R1 Clusters Raw R2
Clusters PF	Clusters PF R1 Clusters PF R2
%PF	%PF R1 %PF R2
Intensity Cycle 1	Intensity Cycle 1 R1 Intensity Cycle 1 R2
% Intensity Cycle 20	% Intensity Cycle 20 R1 % Intensity Cycle 20 R2
% Phasing	% Phasing R1 % Phasing R2
% Prephasing	% Prephasing R1 % Prephasing R2
% Aligned	% Aligned R1 % Aligned R2
% Error Rate	% Error Rate R1 % Error Rate R2

How it Works

The sequencing service may run on Clarity LIMS server in an on-premise environment or on a remote server in a SaaS environment. The service detects event files that the instrument software (RTA) is producing as the run progresses. These event files let the service know where to find the run data.

As the run data are written out and the events come in (Begin Run, Cycle Complete, End Run), the data are matched to the step based on the reagent cartridge ID. This value was entered as the Container Name on the Denature, Dilute and Load Sample 1.2 step. The read-only field values on the Record Details screen are populated accordingly.

Run report generation works as follows.

- Run report generation is asynchronous. The sequencing service does not wait for the report generation to complete before processing the next event file.
- The report is generated by the AUTOMATED - Run Report Generation automation, which is included in the BaseSpaceLIMS-miseqdx-extensions package.
- The AUTOMATED - Run Report Generation automation is configured on the MiSeqDx Run (MiSeqDx) 1.2 step. The default command line is as follows:

```
bash -c "/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/miseqdx/miseqdx-sequencing-report.jar -i
{processLuid} -r {compoundOutputFileLuid0}_RunReport.pdf -l
{compoundOutputFileLuid1}_RunReportLogFile.html
```

- The automation is configured to be invoked by a button on the Record Details screen. However, it is automatically started by the sequencing service when it has finished processing the end run event.

  It is not intended for the user to select the AUTOMATED - Run Report Generation button. The run report is generated and attached to the step automatically.

- An additional shared result-file placeholder is configured on the step. This placeholder is for a new log file—*Illumina Run Report Log File*—that is generated and attached to the step when the report runs.
- The run report can be successfully generated in a hosted deployment of Clarity LIMS where the sequencing service is running on a remote server on the customer network.
- The automation also sets the Report Status field to Completed.

```
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
script:evaluateDynamicExpression -exp 'step.::Report Status:: = ::Completed::' -
t 'false' -log tempLog.html"
```

Following the sequencing run, the run report is automatically generated by the AUTOMATED - Run Report Generation automation. Do not change this name. This name is expected by the sequencing service that captures instrument run results. The base name is stored in the sequenceProcessBaseName database property. If this name is changed without the database property being updated, the 'flow cell ID <-> sequencing step base name' matching will fail.

The Verify Report Status automation prevents movement to the Assign Next Steps screen unless the Report Status field is set to Completed (this is done by the previous automation after the run report is attached to the step).

```
bash -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
script:evaluateDynamicExpression -exp 'if (!step.hasValue( ::Report Status:: )
|| step.::Report Status:: != ::Completed::) { fail(::Cannot advance because
expected files are missing, please wait until the RunReport, RunInfo and
RunParameters files are generated and attached before completing the step.::) }
' -t 'false' -log tempLog.html"
```

User Interaction

> On the Record Details screen, do not select the AUTOMATED - Run Report Generation button. The run report is generated and attached to the step automatically. This process may take a while.

On the Record Details screen of the MiSeqDx Run (MiSeqDx) 1.2 step:

1. Run the instrument. The read-only field values are automatically populated as the instrument runs.
2. After the run has completed, observe the Record Details screen of the MiSeqDx Run (MiSeqDx) 1.2 step for the following.
    - The autopopulated read-only fields.
    - The generated and attached Illumina Run Report and Log File.
    - The attached Run Parameters and Run Info files.
    - The generated and attached Link to Run Folder.
    - QC flags set on each lane.
3. [Optional] In the Files section, upload the Lab Tracking Form.
4. Select Next Steps.
   The Verify Report Status automation should run and allow you to go to the Assign Next Steps screen.
5. On the Assign Next Steps screen, assign the samples to the Variant Calling (MiSeqDx) 1.2 step.
6. Select Finish Step.

Secondary Analysis Output Files Attachment

The Variant Calling (MiSeqDx) 1.2 step attaches secondary analysis output files for each of the samples.

In addition, a Combined Variant Call File, a text file, and a variant call file for each individual sample are automatically captured following the completion of secondary analysis.

How it Works

Following primary analysis, secondary analysis runs on the MiSeqDx instrument. Clarity LIMS detects the completion of secondary analysis and lets the service know where to find the result files. While the EndRun event is processed successfully for primary analysis, the sequence service generates a BeginSecondaryAnalysis event file in the same directory with the `miseqdx.seqservice.eventFileDirectory` property.

An example of how Clarity LIMS shows the location of the secondary analysis event file is:

```
netFolder = D:\Illumina\MiSeqOutput\161017_M99999_0047_FC1234567-
ABCDErunNetworkLocation = /mnt/ussd-dev-mdx01/MiSeqOutput/161017_M99999_0047_
FC1234567-ABCDEeventType = BeginSecondaryAnalysissoftwareType = MRScreationTime
= Thu Oct 20 22:56:32 GMT 2016
```

This event file signals that secondary analysis has started. The sequence service periodically checks if the analysis is complete by looking for the `CompletedJobInfo.xml` file. This file is in the folder specified by the runNetworkLocation parameter of the event file.

The frequency of checking is defined by the `miseqdx.seqservice.synchronizationPeriod` property.

If the completion file appears, the sequence service processes the event. The event is archived in a similar way to how the EndRun event file is handled. If the event completion does not appear within the number days specified by the `miseqdx.seqservice.ignoreUnmatchedContainerIdsWaitDays` property, the service stops monitoring the event file and the file is archived.

Captured Files

The following table describes the Variant Call Format (VCF) files that Clarity LIMS captures and attaches to the step after the secondary analysis.

Table 475 Variant Call Format Files Captured by the LIMS After Secondary Analysis

File Type	Description
Combined Variant Call File	Variant call VCF files for all samples are compressed into a zip file and attached to the step for every run. Available for all 3 assays.
Combined Output Text File	For CF Clinical Sequencing Assay and CF 139-Variant Assay only, a text file containing the summary of variant call information for all samples is attached for every run.
Variant Call File per sample	The general variant call VCF file (not CFTR VCF file) for each sample is attached. Available for all 3 assays.

User Interaction

On the Record Details screen of the Variant Calling (MiSeqDx) 1.2 step:

1. Make sure that the VCF has the correct name based on the sample sheet.
   The file-naming format for the VCF file is SampleName_S#.vcf. In this format, the SampleName is the value in the Sample_Name column of the sample sheet, and # is the sample number determined by the order in which samples are listed in the sample sheet.
2. After secondary analysis has completed, the following files are attached to the step:
   - Combined Variant Call File—a compressed VCF file (zip file) containing all the variant call files, including the CFTR specific VCF files.

- Combined Output Text File—a combined text file containing a summary of all the sample metrics.
- Variant Call File per individual sample

3. QC flags are set on each lane.

Installed Components

The Illumina MiSeqDx Integration RPM package installs the following components.

This integration requires installation of the associated NGS Extensions Package (refer to Release Notes).

Scripts and Files

Files Installed	Location and Description
`configure_extensions_miseqdx_workflow.sh`	`/opt/gls/clarity/config/` Script that imports the integration configuration.
`miseqdx-extensions.jar`	`/opt/gls/clarity/extensions/miseqdx` Jar file containing API-based LIMS extensions used throughout the protocols.
`configure_extensions_miseqdx_sequencingservice.sh`	`/opt/gls/clarity/config/extensions/miseqdx` Script that imports the database properties for the integration.
`log4j2.xml`	`/opt/gls/clarity/extensions/miseqdx/SequencingService/conf` File containing the settings for the sequencing jar logging.
`miseqdx-sequencing-report.jar`	`/opt/gls/clarity/extensions/miseqdx/` Jar file containing API-based LIMS extensions used for report generation.
`miseqdx-sequencing.jar`	`/opt/gls/clarity/extensions/miseqdx/SequencingService` Sequencing service jar file that captures the run results.

Files Installed	Location and Description

`miseqdx_seqservice`	`/opt/gls/clarity/extensions/miseqdx/SequencingService/bin`
	Provides commands for interacting with the sequencing service. Accessible using `systemctl`. For example, use the following command to start the sequencing service:
	`systemctl start miseqdx_seqservice`
	The start keyword can be replaced with stop or status to operate on the sequencing service (eg, `systemctl <start|stop|status> miseqdx_seqservice`)

`run_miseqdx_seqservice`	`/opt/gls/clarity/extensions/miseqdx/SequencingService/bin` Called by `miseqdx_seqservice-v1`, this script performs some basic system validation, then starts the sequencing service.
`MiSeqDxIntegrator.log`	`/opt/gls/clarity/extensions/miseqdx/SequencingService` Log file for Sequencing service.

Database Properties

The following table lists the database properties installed with the Illumina MiSeqDx Integration Package. The following constraints are present when using the database properties:

- Sequencing runs are matched using the flow cell ID and the base name of the sequencing step – MiSeqDx Run (MiSeqDx).
- Do not change this name – it is expected by the sequencing service that captures instrument run results. The base name is stored in the sequenceProcessBaseName database property shown in Table 2. If this name is changed without the database property being updated, the 'flow cell ID <-> sequencing step base name' matching system will fail.
- If necessary, you may modify the step name by editing or adding to the text after the base name portion. This part of the text is not used in the matching system. For example, you could change MiSeqDx Run (MiSeqDx) 1.2 to MiSeqDx Run (MiSeqDx) v1.2.

Several additional properties, each with the ‘99’ suffix appended to their name, are also installed. These properties are intended for use by the Clarity LIMS support team in automated validation tests and are not listed in the table.

Table 476 Database Properties Installed with the Illumina MiSeqDx Integration Package

Property	Description	Default Value
miseqdx.seqservice.ignoreUnmatchedContainerIds	A flag indicating if event files that cannot be matched to flow cells in Clarity LIMS should be archived after a period of time (true), or continually reprocessed (false).	false To prevent the `gls_events` file directory from becoming cluttered, it is recommended that the value of this property is set to 'true'.
miseqdx.seqservice.ignoreUnmatchedContainerIdsWaitDays	The number of days between when the event is created and the event file is archived.	14
miseqdx.seqservice.synchronizationPeriod	Invocation period in seconds.	60
miseqdx.seqservice.sequenceProcessBaseName	Sequencing process type base display name. Partial matching is used to look up the process type.	MiSeqDx Run (MiSeqDx)
miseqdx.seqservice.eventFileDirectory.1	A network location monitored for event files.	/mnt/gls_events
miseqdx.seqservice.netPathPrefixSearch.1	The network directory prefix contained in the event file.	\\nas\network\run_data

Property	Description	Default Value
miseqdx.seqservice.netPathPrefixReplace.1	The mapped network directory mount name on the server used to access the run data directory.	/mnt/run_data
miseqdx.seqservice.eventFileDirectorySuffixes	A list of eventFileDirectory path entries to monitor for event files. The value is one or more comma-separated integers.	99
miseqdx.seqservice.runReportViewsVersion	The current version of the Run Report views in the database. The value 0 represents the state before the views are created. This property is automatically updated by the run report.	0

Property	Description	Default Value
miseqdx.seqservice.netPathPrefixSearchReplaceSuffixes	A list of netPathPrefix search and replace entries for transforming Windows to Linux network paths. The value is one or more comma-separated integers.	99
miseqdx.seqservice.variantCallingProcessBaseName	Sequencing process type base display name. Partial matching is used to look up the process type.	Variant Calling (MiSeqDx)

*It is possible to configure support for multiple, identical seqservice.netPathPrefixSearch property values. For details, refer to *Configure Multiple Identical netPathPrefixSearch Values*.

Reagent Categories/Label Groups

The following reagent categories/label groups are included in the default configuration for the MiSeqDx Integration Package:

- CF 139-Variant Assay Indexes
- CF 139-Variant Assay 8-Sample Indexes
- CF Clinical Sequencing Assay 8-Sample Indexes
- Universal Kit Indexes
- Universal Kit 8-Sample Indexes

Reagent Kits

The following reagent kits are included in the default configuration for the Illumina MiSeqDx Integration Package:

- CF 139-Variant Assay-Oligo Pool
- CF Clinical Sequencing Assay-Oligo Pool
- Custom Oligo Pool
- Elution Buffer
- EtOH
- Extension-Ligation Mix
- Hybridization Buffer
- Index Primers
- Library Beads
- Library Dilution Buffer
- Library Normalization Diluent
- Library Normalization Wash
- Library Storage Buffer
- MiSeqDx Flow Cell - CF 139-Variant Assay
- MiSeqDx Flow Cell - CF Clinical Sequencing Assay
- MiSeqDx Flow Cell - Universal kit
- MiSeqDx SBS Solution (PR2) - CF 139-Variant Assay
- MiSeqDx SBS Solution (PR2) - CF Clinical Sequencing Assay
- MiSeqDx SBS Solution (PR2) - Universal Kit
- NaOH
- PCR Clean-Up Beads
- PCR Master Mix
- PCR Polymerase
- Stringent Wash Buffer
- Universal Wash Buffer

Controls

The following controls are included in the default configuration for the MiSeqDx Integration Package:

- Negative Control for MiSeqDx
- PhiX Internal Control
- Positive Control for MiSeqDx

Container Types

All one-dimensional container types with both numeric rows and numeric columns are supported.

The following container types are included in the default configuration for the MiSeqDx Integration Package:

- MiSeqDx Reagent Cartridge - CF 139-Variant Assay
- MiSeqDx Reagent Cartridge - CF Clinical Sequencing Assay
- MiSeqDx Reagent Cartridge - Universal Kit

Instrument Integration

The following information provides an overview of the steps performed by the Clarity LIMS support team when configuring the instrument for use with the MiSeqDx Integration to Clarity LIMS.

Configure the MiSeqDx as follows.

1. Create a directory on the local computer to hold the batch files. These batch files write event files to the network-attached storage (NAS) shares.
2. Create a directory on the NAS to hold the event files.
3. Modify the software configuration files to call the batch files that create the event files.
4. Update sequencing service default database properties to match the specifics of the installation.

To make sure that your Illumina instrument warranty remains valid, the instrument integration must be performed and maintained by the Clarity LIMS support team. To perform this integration, the Clarity LIMS support team will require direct access to the instrument via WebEx or Remote Desktop while the instrument is idle.

Configuring Instrument Names

We recommend configuring instrument names in Clarity LIMS to track the specific lab instrument on which a run was completed.

This information should be configured on the MiSeqDx Run (MiSeqDx) 1.2 step, and should be set to the same name as is configured in the Illumina sequencing software (ie, this will be value of the Instrument Run ID field described in the *Metadata* section).

When the integration reads the Instrument Run ID field in the run results, it will update the instrument on the step in Clarity LIMS. This update eliminates the need to enter this information manually.

For instructions on configuring instrument names, refer to the Clarity LIMS Core Functionality documentation.

Configuring Sample Sheet Generation

Sample sheet generation occurs on the step prior to the sequencing run—Denature, Dilute and Load Sample 1.2—which is the step where samples are placed on the container that will be loaded in the instrument.

By default, an instrument sample sheet is created for the container loaded during the Denature, Dilute and Load Sample 1.2 step.

Rules and Constraints

This integration operates with the following constraints:

- There must only be a single input on the Denature, Dilute and Load Sample 1.2 step.
- The Reagent Cartridge ID must be unique. There should not be multiple containers in the system with the same name.
- The reagent cartridge ID must be scanned as the Container Name on the Denature, Dilute and Load Sample 1.2 step.
- For MOS sample sheet generation constraints, refer to *Illumina MiSeqDx 1.x Sample Sheet Generation*.

MiSeqDx Integration v1.10 Validation and Troubleshooting

This section explains how to use the MiSeqDx Validation workflow that is included in the Illumina MiSeqDx Integration Package. The workflow allows for simple validation of the following functionality:

- Auto-placement of reagent indexes on the PCR Amplification step
- Generation of a sample sheet file for use with the MiSeqDx Operating Software (MOS) on the Denature, Dilute and Load Sample step

In addition, this section provides tips to help you troubleshoot the system.

All validation steps assume that you have installed the MiSeqDx Integration Package and have imported the default Clarity LIMS configuration. For compatibility information, refer to the *Release Notes*. Only Clarity LIMS v6.2 or later is supported in MiSeqDx Integration Package v1.10 or in subsequent releases.

Instructions for configuring sample sheet generation are provided in *MiSeqDx Integration v1.10 Configuration and User Interaction* on page 2204

Validate Auto-Placement of Indexes and Sample Sheet Generation

After installing and configuring the integration package, and configuring sample sheet generation (refer to *MiSeqDx Integration v1.10 Configuration and User Interaction on page 2204*), follow the steps to activate the MiSeqDx Validation workflow and run samples through it. IPP provides the following validation workflows:

- MiSeqDx Validation (CF 139-Variant Assay) 1.2
- MiSeqDx Validation (CF Clinical Sequencing Assay) 1.2
- MiSeqDx Validation (Universal Kit) 1.2

The following validation steps are based on MiSeqDx Validation (CF 139-Variant Assay) 1.2, where the autoplacement of indexes validation occurs in Run PCR Amplification step and sample sheet generation validation occurs in Run Denature, Dilute and Load Samples step.

Activate Workflow, Add and Assign Samples

1. In the Clarity LIMS web interface, on the Configuration > Workflows screen, activate one of the three Validation workflows:
    - MiSeqDx Validation (CF 139-Variant Assay) 1.2
    - MiSeqDx Validation (CF Clinical Sequencing Assay) 1.2
    - MiSeqDx Validation (Universal Kit) 1.2
2. On the Projects and Samples screen, create a project and assign samples as follows.
    a. Create a test project and add samples to it.
    The project must contain between 9 and 96 samples.
    b. Assign your samples to the MiSeqDx Validation workflow.

Run Extension-Ligation of Bound Oligos Step

1. In Lab View, locate the MiSeqDx Validation protocol. You will see your samples queued for the Extension-Ligation of Bound Oligos step.

Prepare the samples as follows.

2. Add the samples to the Ice Bucket.
3. On the Placement screen, place the samples into the output container.
4. Select Record Details.
5. On the Record Details screen, under Reagent Lot Tracking, select the Extension-Ligation Mix reagent lot used in the step.

   You may need to activate the lot on the Reagents / Reagents and Controls screen.
6. Select Next Steps.
7. On the Assign Next Steps screen, assign the samples to the PCR Amplification step.
8. Select Finish Step.

Run PCR Amplification Step

1. In Lab View, locate the MiSeqDx Validation protocol. You will see your samples queued for the PCR Amplification step.
2. Add the samples to the Ice Bucket.
3. In the Reagent Label Options panel, in the Group of labels list, select CF 139-Variant Assay Indexes (or the equivalent for the workflow you activated).
4. Select Begin Work.
5. On the Placement screen, place the samples into the output container.
6. Select Add Reagents.
   On entry to the Add Reagents screen, the Auto Place Indexes automation is automatically invoked and the reagents are placed on the samples.
7. After placement is complete, a message displays indicating that the index pattern has been applied successfully. Select OK.
8. On the Add Reagents screen, reagents are already placed. Select Record Details.
   On exit from the Add Reagents screen, the Validate Index Placement automation is automatically invoked and index placement is validated.
9. When validation is complete, a message displays indicating that the index pattern has been validated on all samples successfully. Select OK.
10. On the Record Details screen, under Reagent Lot Tracking, select the lots for the following items:
    - Index Primers
    - NaOH
    - PCR Master Mix
    - PCR Polymerase
11. Select Next Steps.

12. On the Assign Next Steps screen, assign samples to the Library Pooling (MiSeqDx) 1.2 step.
13. Select Finish Step.

Run Library Pooling (MiSeqDx) 1.2 Step

1. Return to Lab View and locate the MiSeqDx Validation protocol. You will see your samples queued for the Library Pooling (MiSeqDx) 1.2 step.
   Prepare the samples as follows.
2. Add samples to the Ice Bucket.
3. In the Add Control Samples panel, select PhiX Internal Control. Select Begin Work.
   a. On the Pool Samples screen, create a pool of samples. Select Place Samples.
   b. On the Placement screen, place the pool into the output container. Select Record Details.
   c. Under Reagent Lot Tracking, select the reagent lot used in the step.
   d. Under Step Details, enter the concentration value in the Normalized conc. (nM) field.
4. Select Next Steps.
5. On the Assign Next Steps screen, assign the pool to the Denature, Dilute and Load Samples step.

Run Denature, Dilute and Load Samples Step

1. Return to Lab View and locate the MiSeqDx Validation protocol.
   You will see your pooled samples queued for the Denature, Dilute and Load Sample step.
2. Place the pooled samples as follows.
   a. Add the pool to the Ice Bucket.
      On the Place Samples screen, the Validate Single Input script runs and validates only one pool that is entered into this step as the input sample. If validation fails, Clarity LIMS prompts you that it is only able to proceed with the step with one pool.
   b. On the Placement screen, place the pool into a MiSeqDx reagent cartridge.
   c. Select Record Details.
      On entry to the Record Details screen, a script validates the Container Name and prompts you to scan the reagent cartridge barcode.
3. On the Record Details screen of the Denature, Dilute and Load Sample step, generate the sample sheet as follows.

> All read-only files are autopopulated.

   a. Enter the folder path for the Reference Genomes that will be used for secondary analysis in the GenomeFolder field.
   a. Under Reagent Lot Tracking, select the reagent lots used in the step.
   b. Enter the lots for MiSeqDx Flow Cell - CF 139-Variant Assay and MiSeqDx SBS Solution (PR2) - CF 139-Variant Assay.

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c. In the Step Details section, review the step parameters.
d. Select Generate MiSeqDx Sample Sheet to generate the MiSeqDx sample sheet.
e. Clarity LIMS attaches the generated sample sheet and log file to placeholders in the Files area of the Record Details screen. Download the files and validate their format and content.
f. [Optional] In the Files section, upload the Lab Tracking Form to the appropriate placeholder.

4. Select Next Steps.
5. On the Assign Next Steps screen, assign the samples to the MiSeqDx Run (MiSeqDx) 1.2 step.

Validate Creation of Event Files

Validating the creation of the event files confirms the following:

- Your DESTINATION_PATH is correctly configured.
- The instrument computer can access and write to the DESTINATION_PATH.
- There are no syntax errors in the Clarity LIMS batch file.

Follow the steps below to confirm that event files are created by the batch file in the destination path. The steps assume that the default configuration has been successfully imported.

1. In `C:\Illumina\gls`, double-click `gls_event_mos_rta.bat`.
2. Confirm that an empty event file appears in the configured DESTINATION_PATH.
3. Manually invoke the `gls_event_mos.bat` file.
   The file produces an output similar to the following example:

```
cycleNumber = 318
runFolder = "D:\Illumina\MiSeqTemp\161030_M99999_0056_FC1234567-ABCDE"
netFolder = "D:\Illumina\MiSeqOutput\161030_M99999_0056_FC1234567-ABCDE"
readType = 4
eventType = EndRun
softwareType = MOS
finishDate = 2016-10-30
```

Troubleshooting

If an automation trigger does not appear to run its corresponding scripts, see:

- *Troubleshooting Automated Informatics* in the LIMS documentation in the Automated Informatics section.
- *Troubleshooting EPP / Automation* in the API > Overview > API Advanced Topics section.

If an error occurs that does not provide direction on how to proceed, troubleshoot the error as follows.

1. Confirm the version of the installed Illumina MiSeqDx Integration Package by running the `rpm -qa | grep -i miseqdx` command from the server console.
2. If the error is related to retrieving run results data, review the MiseqDxIntegrator.log.

3. Contact the Clarity LIMS Support team, supplying the relevant information from the troubleshooting already performed.

MiSeqDx Integration v1.10 Installation

The Illumina MiSeqDx Integration Package v1.10 supports MiSeqDx instruments running in Diagnostic mode. For MiSeqDx instruments running in Research mode, install the MiSeq Integration Package. For more information, refer to *MiSeq Integration v8.2 Installation* on page 2007.

Integration Overview

MiSeqDx Integration v1.10 includes the following new features:

- Support for Clarity LIMS v6.2 and Oracle Linux. For more information on compatible Oracle Linux versions, refer to *MiSeqDx Integration v1.10 Release Notes* on page 2244.
- The Validate Single Input automation in the Denature, Dilute and Load Sample step.

Compatibility

MiSeqDx Integration v1.10 is compatible with the following software:

- Clarity LIMS v6.2 and later
- Secret Util v1.0 and later
- IPP v2.6 and later

Prerequisites

MiSeqDx Integration v1.10 has the following prerequisites:

- Mount run data network-attached storage (NAS) share
- IPP is installed

Prerequisite 1: Mount Run Data NAS Share

Mounting the NAS share of run data are needed to capture and generate files associated with the sequencing run. To mount NAS shares that contain data from the Clarity LIMS server, use Read/Write privileges as the glsjboss user. The following data can be mounted to the NAS share:

- Run data (eg, `\\network-storage\run_data\`)
- Clarity LIMS-created events triggered by the End Run event of the Illumina sequencing run (eg, `\\network-storage\illumina\gls_events\`)

With Read access, the Clarity LIMS server reads the following information in individual sequencing run data folders:

- Run information metadata from `<runFolderRoot>/RunInfo.xml and <runFolderRoot>/RunParameters.xml`
- Run statistics from `<runFolderRoot>/InterOp/*.bin`
- Text and VCF files from the Alignment folder at `<runFolderRoot>/Data/Intensities/BaseCalls/Alignment<#>/*.vcf`

> CFTR-specific files with the extension cftr.vcf are ignored.

The Clarity LIMS server generates the following files and information locally and stores them in Clarity LIMS:

- Sample sheet (CSV file, eg, `SampleSheet.csv`)
- Run report (PDF file, eg, `runreport.pdf`)
- Run folder root link

The Clarity LIMS server copies and stores the following files from individual sequencing run data folders in Clarity LIMS:

- `<runFolderRoot>/RunInfo.xml`
- `<runFolderRoot>/RunParameters.xml`
- `<runFolderRoot>/first_base_report.htm`
- `<runFolderRoot>/Data/Intensities/BaseCalls/Alignment<#>/*.vcf` (applicable to all protocols)
- `MiSeqDxCF139VariantAssay.txt` (for CF 139-Variant assay)
- `MiSeqDxCFClinicalSequencingAssay.txt` (for CF Clinical Sequencing assay)

Prerequisite 2: IPP Installation

MiSeqDx Integration Package v1.10 depends on the QC Protocols configuration provided in IPP (v2.6 and later) for Clarity LIMS v6.2 and later.

If the base configuration is not installed, then install it on the Clarity LIMS server that is being used for the MiSeqDx integration. For details on IPP v2.6 installation and configuration, refer to the *Illumina Preset Protocols* documentation.

If you are upgrading the base configuration, make sure that the IPP package is compatible with the version of Clarity LIMS you are installing (in this case, IPP v2.6 for Clarity LIMS v6.2).

If you do not have QC Protocols, then install them as follows.

1. As a glsjboss user, run the following command to view the complete list of IPP workflows:

```
/opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o list
```

2. To install the dependent QC Protocols, run the following command:

```
/opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o install QC_
Protocols.qc-protocols
```

Installation

MiSeqDx Integration v1.10 supports both on-premise and cloud integrations. This integration is distributed as the following RPM packages:

- BaseSpaceLIMS-miseqdx-extensions
- BaseSpaceLIMS-miseqdx-sequencing-service

The BaseSpaceLIMS-miseqdx-extensions RPM installs the following items:

- Protocols and workflows
- Database properties that configure the service
- Placement pattern files that determine reagent index assignment
- `miseqdx-extensions.jar`
- `miseqdx-sequencing-report.jar`

The BaseSpaceLIMS-miseqdx-sequencing-service RPM installs the following items:

- If not found, user configuration (the glsjboss user and the glsjdk8 and claritylims user groups)
- If not installed, Java 8
- Bash scripts to run the miseqdx_seqservice
- `miseqdx-sequencing.jar`
- The `gls_events_mos_rta.bat` batch file that configures RTA
- Smoke test directories

On-Premise Installation

Use the following instructions to install the BaseSpaceLIMS-miseqdx-extensions and BaseSpaceLIMS-miseqdx-sequencing-service RPMs on the Clarity LIMS server.

Install the RPMs

1. On the Clarity LIMS server, log in as the root user.
2. Run the following yum commands to install the RPMs:
   ```
   yum install BaseSpaceLIMS-miseqdx-extensions
   yum install BaseSpaceLIMS-miseqdx-sequencing-service
   ```
3. Enter `y` to confirm that you want to proceed with the RPM installations.
   After confirmation, the following scripts and files are installed:
   - `configure_extensions_miseqdx_workflow.sh`
   - `configure_extensions_miseqdx_sequencingservice.sh`

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- `miseqdx-extensions.jar` (contains the sample sheet generation and other scripts)
- `miseqdx-sequencing-report.jar` (generates the sequencing run report)

These files are installed at the following locations:

- `/opt/gls/clarity/config/`
- `/opt/gls/clarity/extensions/miseqdx`
- `/opt/gls/clarity/extensions/miseqdx/SequencingService`

Import Workflow Configurations for MiSeqDx

If you are upgrading from an earlier version of the MiSeqDx integration package and the system is configured with the configure_extensions_miseqdx_workflow.sh and configure_extensions_miseqdx_sequencingservice.sh scripts, go to *Configure the Database Service Properties* on page 2237.

1. When prompted by the RPM instructions to import the workflow configuration, run the following command as the glsjboss user:
   ```
   bash /opt/gls/clarity/config/configure_extensions_miseqdx_workflow.sh
   ```
2. When prompted by the RPM instructions to configure the sequencing service (which includes database properties with default values set for the integration), run the following command as the glsjboss user:
   ```
   bash /opt/gls/clarity/config/configure_extensions_miseqdx_
   sequencingservice.sh
   ```

Configure the Database Service Properties

For more information on the properties that must be configured, refer to *Installed Components* on page 2223.

Start the Sequencing Service

1. Run the following command to start the sequencing service:
   ```
   systemctl start miseq_seqservice-v8
   ```

Cloud Installation

The BaseSpaceLIMS-miseqdx-extensions RPM must be installed on the Clarity LIMS server. The BaseSpaceLIMS-miseqdx-sequencing-service RPM can be installed remotely on another server within the network.

Specifications

The following hardware, operating system, and network specifications must be met to install the BaseSpaceLIMS-miseqdx-sequencing-service RPM:

- Hardware requirements:
  - 64-bit processor (dual core 2.0 GHz)

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  - OS requirements, plus at least an additional 512 MB RAM
  - A minimum of 5 GB of hard disk space
- Operating system requirements:
  - Oracle Linux (for compatibility, refer to *MiSeqDx Integration v1.10 Release Notes* on page 2244)
- Network requirements:
  - SSL access to the Clarity LIMS server from the network
  - A mounted network folder where the sequencing runs are written

Install the RPM

1. On the applicable server, log in as the root user.
2. Run the following yum command to install the RPM:
   ```
   yum install BaseSpaceLIMS-miseqdx-extensions
   ```
3. Enter `y` to confirm that you want to proceed with the RPM installations.
   After confirmation, the following scripts and files are installed:
   - `configure_extensions_miseqdx_workflow.sh`
   - `configure_extensions_miseqdx_sequencingservice.sh`
   - `miseqdx-extensions.jar` (contains the sample sheet generation and other scripts)
   - `miseqdx-sequencing-report.jar` (generates the sequencing run report)

   These files are installed at the following locations:
   - `/opt/gls/clarity/config/`
   - `/opt/gls/clarity/extensions/miseqdx`
   - `/opt/gls/clarity/extensions/miseqdx/SequencingService`

Import Workflow Configurations for MiSeqDx

If you are upgrading from an earlier version of the MiSeqDx integration package and the system is configured with the configure_extensions_miseqdx_workflow.sh and configure_extensions_miseqdx_sequencingservice.sh scripts, go to *Configure the Database Service Properties* on page 2237.

1. When prompted by the RPM instructions to import the workflow configuration, run the following command as the glsjboss user:
   ```
   bash /opt/gls/clarity/config/configure_extensions_miseqdx_workflow.sh
   ```
2. When prompted by the RPM instructions to configure the sequencing service (which includes database properties with default values set for the integration), run the following command as the glsjboss user:
   ```
   bash /opt/gls/clarity/config/configure_extensions_miseqdx_
   sequencingservice.sh
   ```


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Configure the Database Service Properties

For more information on the properties that must be configured, refer to *Installed Components* on page 2223.

Install Sequencing Service RPM on Remote Server

1. On the applicable server, log in as the root user.
2. Run the following yum command to install the RPM:
   ```
   yum install BaseSpaceLIMS-miseqdx-sequencing-service
   ```
3. Enter `y` to confirm that you want to proceed with the RPM installation.

Copy API Connection Properties File from the Clarity LIMS Server to the Remote Server

1. Make sure that the extensions RPM is installed on the Clarity LIMS server.
2. Run the following command to generate the `integration.properties` API connection properties file:
   ```
   java -jar /opt/gls/clarity/extensions/miseqdx/miseqdx-extensions.jar
   script:com.genologics.integrations.sequencing.generate_integration_property_
   file
   ```
3. Copy the `integrations.properties` file from the Clarity LIMS server to the following location on the remote server:
   ```
   /opt/gls/clarity/extensions/miseqdx/SequencingService/conf
   ```

Start the Sequencing Service

1. Run the following command to start the sequencing service:
   ```
   systemctl start miseq_seqservice
   ```

Workflows, Protocols, and Steps Installed

MiSeqDx Integration v1.10 installs the following protocols:

- CF 139-Variant Assay Library Prep 1.2
- CF Clinical Sequencing Assay Library Prep 1.2
- Universal Kit Library Prep 1.2
- Illumina SBS MiSeqDx (CF 139-Variant Assay) 1.2
- Illumina SBS MiSeqDx (CF Clinical Sequencing Assay) 1.2
- Illumina SBS MiSeqDx (Universal Kit) 1.2

The integrations also install the following validation protocols that are included in a workflow with the same name:

- MiSeqDx Validation (CF 139-Variant Assay) 1.2
- MiSeqDx Validation (CF Clinical Sequencing Assay) 1.2
- MiSeqDx Validation (Universal Kit) 1.2

For descriptions of the protocol and the steps, refer to *MiSeqDx Integration v1.10 Configuration and User Interaction* on page 2204. For instructions on using the MiSeqDx validation workflows to validate the automated sample sheet generation, refer to *MiSeqDx Integration v1.10 Validation and Troubleshooting* on page 2229.

Instrument Software

The instrument software is divided into the following modules:

- MiSeqDx Operating Software (MOS)—Controls the instrument operation, including various configuration settings. This software is installed and runs on the instrument.
- MiSeqDx Reporting Software (MRS)—Performs the following secondary analysis functions:
  - Demultiplexing
  - Alignment
  - Variant calling
  - Report generation

  The specific functions that are supported vary by the kit. This software is installed on or off the instrument.
- Real-Time Analysis (RTA)—Performs image processing and base calling (primary analysis). The software makes sure that data files are created and copied to the final destination folder and is installed and runs on the instrument.
- Illumina User Management (IUM)—Contains a user database file that is used with the MiSeqDx instrument. This file controls user passwords and privileges for MOS.

For more information on the MiSeqDx software, refer to the MiSeqDx documentation at support.illumina.com.

Instrument Integration

Illumina provides a supported mechanism for using custom scripts on key events during a sequencing run. The Clarity LIMS support team has created batch files that plugs into these events. When the batch file is used, it reads the event information and writes it in a TXT event file at the same network share location that the instrument uses to write the run data. Another process running on the server where the sequencing service RPM is installed receives the event files and takes the appropriate actions.

The sequencing service monitors the following events (the actual event names may be different):

- End Run—This event is used to update the sequencing steps in Clarity LIMS, captures key process data and files, and parses run statistics for output custom fields.
- Begin Secondary Analysis—Indicates that secondary analysis in the MRS has started so that the sequencing service can start to monitor for results. After secondary analysis is complete, the VCF files are uploaded to Clarity LIMS.

Configure Batch Files

When the instrument is running, the final destination for the run data are a network storage path. The software is configured with a network storage path root (eg, `\\network-storage\illumina\`). Each sequencing run generates a unique run ID, which is appended to create a unique data run directory (eg, `\\network-storage\illumina\110419_InstrumentName_0001_ARG1234567`).

The Clarity LIMS batch files must be configured to write to a directory within the network storage path root. This directory is typically named `gls_events`, but the directory name can be different as long as no spaces are used.

To avoid inadvertently removing or overwriting the batch file when updating the instrument software, the batch file can be placed in its own directory on the instrument computer.

Before configuring the batch files, do the following:

1. Back up the `MiSeq.Configuration.xml` configuration file to `C:\Illumina\RTA\Configs\`.
2. Make sure that the instrument is idle.
3. Shut down MOS.
4. Set up the directory structure as follows.
   a. Create a directory (`C:\Illumina\gls` is recommended) on the local PC to hold the batch file.

   > Because of Windows 10 software restriction policies, this folder must be under C:\Illumina. Otherwise, the script is prohibited from running.

   b. Create a directory (eg, `gls_events`) on the NAS to hold the event files.
5. Deploy the batch file as follows.
   a. On the server where the sequencing service RPM is installed, copy the batch file from `/opt/gls/clarity/extensions/miseqdx/InstrumentIntegrations` to `C:\Illumina\gls` on the local computer.
   b. If necessary, create the `gls_events_mos_rta.bat` batch file in `C:\Illumina\gls`. For more information, contact Illumina Support.
   c. From the command prompt, list the contents on the `C:\Illumina\gls` directory using the following command:
   ```
   dir C:\Illumina\gls\
   ```
   Make sure that the name of the batch file you created does not contain any special (hidden) characters.

Configure the batch file as follows.

1. Determine the site or instrument specific network storage path root.
2. Change the DESTINATION_PATH line to use the storage path root and the name of the event file directory.

> Make sure to include the trailing \ in the DESTINATION_PATH line. Refer to the following example:
>
> ```
> set DESTINATION_PATH=\\network-storage\illumina\gls_events\
> ```

3. Copy the DESTINATION_PATH and paste it into the Windows Explorer address bar.
4. Make sure that the network location is accessible and that it opens from the instrument.

Configure RTA

Update the MOS configuration files as follows.

1. Turn off the instrument and restart the instrument computer.
2. Using Task Manager, make sure that MOS is not auto-launched by Windows.
3. [Optional] If MOS is auto-launched, remove it from the auto-launch list and restart the computer.
4. Edit the file to connect to the RTA End Run event as follows.
   a. Open the `MiSeq.Configuration.xml` file at `C:\Illumina\RTA\Configs\`.
   b. Update the following content within the <RTAConfiguration> tags:
   ```
   <ProcessCompleteEventFile>C:\Illumina\gls\gls_event_mos_
   rta.bat</ProcessCompleteEventFile>
   ```
5. Save and close the edited file.
6. Validate the file as follows.
   a. Open `MiSeq.Configuration.xml` configuration file in Explorer to perform some of the XML validation.
   b. To further validate the file, run the following command from the Command Prompt:
   ```
   C:\Illumina\RTA\RTA.exe "."
   configFile="C:\Illumina\RTA\Configs\MiSeq.Configuration.xml"
   Processing.WorkProviderTypes="OfflineBased" processedfolder="."
   ```
   If the validation is successful, the following message displays:
   ```
   Error: No run info file found in input directory .\RunInfo.xml
   ```
   If the configuration files contain an error, the command returns specifics about the problem. The following example shows an error that occurs when a <GLS> key is added to the file at line 83:
   ```
   Error: While loading Configuration
   "c:\illumina\rta\configs\MiSeq.Configuration.xml"
   There is an error in XML document <83, 5>
   Error loading xml file: Unknown node 'GLS' at line 83 pos 5
   ```
7. Start MOS.
8. Open the `MiSeq.Configuration.xml` file and make sure that the changes were saved.

Validation

Sample Sheet Generation

For instructions on how to validate the automated sample sheet generation, refer to *MiSeqDx Integration v1.10 Validation and Troubleshooting* on page 2229.

Manual Invocation of Event Files

For instructions on validating the creation of event files, refer to *MiSeqDx Integration v1.10 Validation and Troubleshooting* on page 2229.

Instrument Sequencing Run

The instrument sequencing run test validates that the Clarity LIMS batch file is connected properly and invoked on the instrument events. Before validating the batch file, make sure that you have the following prerequisites are met:

- You have access to the NAS share.
- The default configuration has been successfully imported.
- Manual invocation of the event files has been validated. This validation checks for the following information:
  - The DESTINATION_PATH is configured correctly.
  - The instrument computer can access and write to the DESTINATION_PATH.
  - There are no syntax errors in the Clarity LIMS batch file.

For more information on event file validation, refer to *MiSeqDx Integration v1.10 Validation and Troubleshooting* on page 2229.

The sequencing service processes and archives event files, which can cause validation issues while the service is running. You can make the following changes to avoid losing the event files that you are attempting to validate:

- Modify the FINAL_EXTENSION value in the Clarity LIMS batch file so that the file extension is .test instead of .txt. The service only processes and archives TXT files. Make sure that you change FINAL_EXTENSION back to .txt after manual validation.
- Monitor the `MiSeqDxIntegrator.log` file, which logs the file name and contents of each event file that is processed.

Validate the sequencing run as follows.

1. During the run, monitor the contents of the gls_events directory.
2. After the run is completed and the RTA completes primary analysis, make sure that a final EndRun event displays (eg, `event-EndRun-11043279.txt`).

MiSeqDx Integration v1.10 Manual Upgrade

MiSeqDx Integration v1.10 includes the MiSeqDx v1.2 workflow upgrade. This upgrade is typically done through the RPM. To upgrade the workflow manually, create the Validate Single Input automation and update its trigger information as follows.

Before updating the automations, make sure the MiSeqDx v1.1 workflow is installed.

Create Validate Single Input Automation

1. In Clarity LIMS, under Configuration, select the Automation tab.
2. Select New Automation.
3. Create an automation called Validate Single Input.
4. In the Command Line field, add the following string:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:validateSampleCount -min 1 -max 1"
```

5. Under Automation Use, enable this automation for the Denature and Dilute master steps on all variant workflows.

Change Trigger Point for the Validate Single Input Automation

1. In Clarity LIMS, under Configuration, select the Lab Work tab.
2. Find the Denature and Dilute steps in all variant workflows.
3. In the Automation section of each step, update the Trigger Location and Trigger Style for the Validate Single Input automation as follows.
   - Trigger Location—Step
   - Trigger Style—Automatic upon entry

MiSeqDx Integration v1.10 Release Notes

These Release Notes describe the key changes to software components for the Clarity LIMS MiSeqDx Integration Package version 1.10. This is an optional software update.

Compatibility

This version of the integration package is compatible with the following software:

- Clarity LIMS v6.2 or later
- NGS Extensions Package v5.23 or later
- The following operating systems:
  - RedHat Enterprise Linux (64-bit) v8.8
  - Oracle Linux (64-bit) v8.8

New Features

- Updated Java and third-party dependency libraries.
- Updated Groovy to v3.0.7.

Defects Fixed

- Fixed the MiSeqDx Run Type custom field value not displaying in the MiSeqDx Run step in the MiSeqDx Validation (CF 139-Variant Assay) protocol.

- Fixed the MiSeqDx run report not displaying workflow information.
- The Denature and Dilute step now only allows a single pool to be added to the Ice Bucket when you enter the step.
- Fixed the SQL Injection (CVE-2022-31197) and Information Disclosure (CVE-2020-15522) vulnerabilities.

Known issues

None

MiSeqDx Integration v1.9 Configuration and User Interaction

The Illumina MiSeqDx Integration Package v1.9 supports the integration between Clarity LIMS and MiSeqDx instruments. The integration allows for automated tracking of an Illumina sequencing run in Clarity LIMS and includes the following functionality:

- Tracking instrument run status.
- Generating a run report.
- Capturing and parsing run statistics.
- Uploading variant call files to the LIMS.

In addition, this integration provides automated generation of a sample sheet file for use with the MiSeq Operating Software (MOS).

This documentation describes the integration between Clarity LIMS and the MiSeqDx system. It includes information about protocols and automations, configuration options, installed components, and rules and constraints.

Integration Features

- MiSeqDx RPM that supports Diagnostic (Dx) mode.
- MiSeq RPM that supports Research (RUO) mode.
- Preconfigured Clarity LIMS protocols that map to library prep and sequencing lab protocols and instrument runs.
- Preconfigured protocols that allow for validation of the sample sheet generation and index placement functionality.
- Automatic generation of:
  - A sample sheet file for use with the MOS (Diagnostic mode) instrument software.
  - A run report, which includes the current run statistics.
- Automated tracking of the following information in Clarity LIMS:

  - Progress and metrics of a MiSeqDx sequencing run
  - Per-instrument sequencing runs
  - Sequencing run parameters
  - Real-Time Analysis (RTA) run directory location and other run specific information
- The ability to monitor the run status (cycle number) across multiple instruments from within Clarity LIMS.
- The ability to attach Variant Call Format (VCF) files to Clarity LIMS.

Protocols

The following protocols are included in MiSeqDx Integration Package v1.9:

- CF 139-Variant Assay Library Prep 1.1
- CF Clinical Sequencing Assay Library Prep 1.1
- Universal Kit Library Prep 1.1
- SBS MiSeqDx (CF 139-Variant Assay) 1.1
- SBS MiSeqDx (CF Clinical Sequencing Assay) 1.1
- SBS MiSeqDx (Universal Kit) 1.1

There are three validation protocols. Each protocol is included in a workflow with the same name. The protocols are as follows.

- MiSeqDx Validation (CF 139-Variant Assay) 1.1
- MiSeqDx Validation (CF Clinical Sequencing Assay) 1.1
- MiSeqDx Validation (Universal Kit) 1.1

In Assay workflows, include a QC validation protocol between the Library Prep and Illumina SBS protocols. The default workflow does not include a QC protocol.

Each validation protocol includes the following steps from the Library Prep and SBS MiSeqDx protocols:

- Extension-Ligation of Bound Oligos (Library Prep step)
- PCR Amplification (Library Prep step)
- Library Pooling (MiSeqDx) (SBS MiSeqDx step)
- Denature, Dilute and Load Sample (SBS MiSeqDx step)
- MiSeqDx Run (MiSeqDx) (SBS MiSeqDx step)
- Variant Calling (MiSeqDx) (SBS MiSeqDx step)

For instructions on using the MiSeqDx Validation (CF 139-Variant Assay) 1.0 protocol to validate automated sample sheet generation, see *MiSeqDx Integration v1.9 Validation and Troubleshooting* on page 2270.

Automations

The following table lists the automations included in this integration, and the steps on which they are configured.

Table 477 MiSeqDx Integration Default Automations

Protocol	Steps & Automations
• CF 139-Variant Assay Library Prep 1.1 • Universal Kit Library Prep 1.1	On PCR Amplification (CF 139-Variant Assay / Universal Kit) 1.1 step: • Auto Place Indexes • Validate Index Placement
• Illumina SBS MiSeqDx (CF 139-Variant Assay) 1.1 • Illumina SBS MiSeqDx (CF Clinical Sequencing Assay) 1.1 • Illumina SBS MiSeqDx (Universal Kit) 1.1	On Library Normalization (MiSeqDx) 1.1 step: • Create Normalization CSV
• Illumina SBS MiSeqDx (CF 139-Variant Assay) 1.1 • Illumina SBS MiSeqDx (CF Clinical Sequencing Assay) 1.1 • Illumina SBS MiSeqDx (Universal Kit) 1.1 • MiSeqDx Validation (CF 139-Variant Assay) 1.1 • MiSeqDx Validation (CF Clinical Sequencing Assay) 1.1 • MiSeqDx Validation (Universal Kit) 1.1	On Denature, Dilute and Load Sample (CF 139-Variant Assay / CF Clinical Sequencing Assay / Universal Kit) 1.1 step: • Generate MiSeqDx Sample Sheet • Validate Container Name
• Illumina SBS MiSeqDx (CF 139-Variant Assay) 1.1 • Illumina SBS MiSeqDx (CF Clinical Sequencing Assay) 1.1 • Illumina SBS MiSeqDx (Universal Kit) 1.0 • MiSeqDx Validation (CF 139-Variant Assay) 1.1 • MiSeqDx Validation (CF Clinical Sequencing Assay) 1.1 • MiSeqDx Validation (Universal Kit) 1.1	On MiSeqDx Run (MiSeqDx) 1.1 step: • AUTOMATED - Run Report Generation • Verify Report Status

Terminology

As of Clarity LIMS v5, the term user-defined field (UDF) has been replaced with custom field in the user interface. However, the API resource is still called UDF.

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There are two types of custom fields:

- Master step fields—Configured on master steps. Master step fields only apply to the following:
  - The master step on which the fields are configured.
  - The steps derived from those master steps.
- Global fields—Configured on entities (eg, submitted sample, derived sample, measurement, etc.). Global fields apply to the entire Clarity LIMS system.

Library Prep Protocols

This section discusses the Library Prep protocols, steps, and automations included in the MiSeqDx v1.9 Integration Package.

Index Placement and Validation Automations

This section discusses the index placement and validation automations configured on the PCR Amplification 1.1 Library Prep steps.

The example workflow uses the CF 139-Variant Assay Library Prep 1.1 protocol. However, important considerations related to the Universal Kit Library Prep 1.1 and CF Clinical Sequencing Assay Library Prep 1.1 protocols are noted.

By default, in the CF 139-Variant Assay Library Prep 1.1 and the Universal Kit Library Prep 1.1 protocols, the PCR Amplification 1.1 step includes two automations. Both automations invoke the `place_indexes` script with different options.

- Auto Place Indexes—Automatically invoked on entry to the Add Reagents screen.
- Validate Index Placement—Automatically invoked on exit from the Add Reagents screen.

A list of reagents installed with this configuration is provided in *Installed Components* on page 2263.

Good laboratory practices mandate that a positive control DNA sample and a negative (no-template) control sample are included in every run. The positive control DNA sample should be a well-characterized sample with a known CFTR mutation.

How it Works

On entry to the Add Reagents screen

The Auto Place Indexes automation is invoked. This in turn invokes the `place_indexes` script, which places reagent indexes into the container based on the pattern defined in a specified index placement pattern file. (The Step Automation Details table that follows shows the pattern file names and their location.)

Reagent indexes are assigned to each sample in the destination container according to the specified index placement pattern file. Note the following:

- The automatic index placement pattern for Universal Kit can be modified.

- The automatic index placement pattern for CF 139-Variant Assay should not be modified.
- If an 8-sample reagent category / label group is selected (see step 1 of *User Interaction* section below), index placement must be performed manually.

  Index placement must be performed manually for the CF Clinical Sequencing Assay Library Prep 1.1 protocol, which only uses 8-sample reagent categories / label groups.

- Upon completion of the reagent index placement, a success message displays. The user selects OK to close the message. Indexes with the pattern specified in the placement file are then assigned to the samples.

On exit from the Add Reagents screen

The Validate Index Placement automation invokes the `place_indexes` script once again, this time to validate the index placement:

- If the index placement is user-modifiable (Universal Kit only), any changes made by the user are preserved.
- If the index placement cannot be modified (CF 139-Variant Assay), any changes made by the user are disregarded and the automatic index placement is restored.

Step Automation Details

The following table shows the automation commands configured on the Auto Place Indexes and Validate Index Placement automations during the PCR Amplification (CF 139-Variant Assay) 1.1 step.

Table 478 PCR Amplification 1.1 Step Automation Details

Automation	Trigger	Command
PCR Amplification (CF 139-Variant Assay) 1.1 step:		
Auto Place Indexes	On entry to Add Reagents screen	`bash -l -c "/opt/gls/clarity/bin/java -cp /opt/gls/clarity/extensions/ngs-common/v5/EPP/PlacementHelper.jar place_indexes -i {stepURI:v2} -u {username} -p {password} -f /opt/gls/clarity/extensions/conf/miseqdx/placementpatterns/placement_pattern_index_cf139_01.tsv -ic 'CF 139-Variant Assay 8-Sample Indexes' -locked true"`

Automation	Trigger	Command
Validate Index Placement	On exit from Add Reagents screen	`bash -l -c "/opt/gls/clarity/bin/java -cp /opt/gls/clarity/extensions/ngs-common/v5/EPP/PlacementHelper.jar place_indexes -i {stepURI:v2} -u {username} -p {password} -f /opt/gls/clarity/extensions/conf/miseqdx/placementpatterns/placement_pattern_index_cf139_01.tsv -ic 'CF 139-Variant Assay 8-Sample Indexes' -locked true -fc true"`
PCR Amplification (Universal Kit) 1.1 step:		
Auto Place Indexes	On entry to Add Reagents screen	`bash -l -c "/opt/gls/clarity/bin/java -cp /opt/gls/clarity/extensions/ngs-common/v5/EPP/PlacementHelper.jar place_indexes -i {stepURI:v2} -u {username} -p {password} -f /opt/gls/clarity/extensions/conf/miseqdx/placementpatterns/placement_pattern_index_universal_01.tsv -ic 'Universal Kit 8-Sample Indexes'"`
Validate Index Placement	On exit from Add Reagents screen	`bash -l -c "/opt/gls/clarity/bin/java -cp /opt/gls/clarity/extensions/ngs-common/v5/EPP/PlacementHelper.jar place_indexes -i {stepURI:v2} -u {username} -p {password} -f /opt/gls/clarity/extensions/conf/miseqdx/placementpatterns/placement_pattern_index_universal_01.tsv -ic 'Universal Kit 8-Sample Indexes' -fc true"`

User Interaction

The section describes the user interaction involved in running samples through the PCR Amplification (CF 139-Variant Assay) 1.1 step.

1. Run the PCR Amplification (CF 139-Variant Assay) 1.1 step. Add samples to the Ice Bucket and select Reagent Label Options.
2. Select one of the following options: SME reviewer: Clarification needed on this step. Please see note on Confluence source doc.
   - For manual placement of indexes with less than eight samples, select CF 139-Variant Assay 8-Sample Indexes.
   - For automatic placement of indexes with more than eight samples, select CF 139-Variant Assay Indexes.
3. Select Begin Work.

4. On the Record Details screen, select Add Reagents.
5. A message box displays, notifying you that a custom script is in progress. Upon script completion, a success message displays. Select OK to proceed.
6. Make sure the indexes with the pattern specified in the placement file are assigned to samples and displayed.

> If you are running the Universal Kit protocol, you may modify the index placement. If you are running the CF 139-Variant Assay protocol, index placement is not modifiable. If you attempt to modify the placement pattern, your changes will be reverted on exit from the Add Reagents screen and the automatic placement will be restored.

7. Select Next Steps.
   Clarity LIMS does the following:
   - Validates the index placement.
   - Reverts any changes made to index placement in the CF 139-Variant Assay protocol.
8. A success message displays. Select OK to proceed.

Step 1: Hybridization of Oligo Pool (CF 139-Variant Assay) 1.1

The following process is used to hybridize the oligo pool.

1. Add samples to Ice Bucket.
2. Add Negative Control and Positive Control samples to Ice Bucket.

   > Good laboratory practices mandate that a positive control DNA sample and a negative (no-template) control sample are included in every run. The positive control DNA sample should be a well-characterized sample with a known CFTR mutation.

3. Select Begin Work.
4. On the Placement screen, select all samples and place in container. Select Record Details.
5. On the Record Details screen, under Reagent Lot Tracking, set the following values:
   - gDNA Concentration (ng/uL): 50 ng/ul
   - gDNA Volume (uL): 5 uL
   - Select the reagent lots for CF 139-Variant Assay-Oligo Pool and Hybridization Buffer.
6. Select Next Steps.
7. Select Removal of Unbound Oligos (CF 139-Variant Assay) 1.1 as the next step. Select Apply.
8. Select Finish Step.

Step 2: Removal of Unbound Oligos (CF 139-Variant Assay) 1.1

The process below is used to remove unbound oligos.

1. Add samples to Ice Bucket.

2. Select Begin Work.
3. On the Placement screen, select all samples and place in container.
4. Select Record Details.
5. On the Record Details screen, under Reagent Lot Tracking, select the reagent lots for Stringent Wash Buffer and Universal Wash Buffer.
6. Select Next Steps.
7. Select Extension-Ligation of Bound Oligos (CF 139-Variant Assay) 1.1 as the next step. Select Apply.
8. Select Finish Step.

Step 3: Extension-Ligation of Bound Oligos (CF 139-Variant Assay) 1.1

The process below is used to add reagents to the Extension-Ligation Mix field.

1. Add samples to Ice Bucket. Select Begin Work.
2. On the Placement screen, select all samples and place in container.
3. Select Record Details.
4. On the Record Details screen, under Reagent Lot Tracking, select the reagent lots for Extension-Ligation Mix.
5. Select Next Steps.
6. Select PCR Amplification (CF 139-Variant Assay) 1.1 as the next step. Select Apply.
7. Select Finish Step.

Step 4: PCR Amplification (CF 139-Variant Assay) 1.1

1. Add samples to Ice Bucket.
2. Select one of the following Reagent Label Options:
    - If you are working with eight samples or less, select CF 139-Variant Assay 8-Sample Indexes.
    - If you are working with more than 8 samples, select CF 139-Variant Assay Indexes.
3. Select Begin Work.
4. On the Placement screen, select all samples and place in container. Select Add Reagents.
   On entry to the Add Reagents screen, the Auto Place Indexes automation is invoked and the reagents are placed on the samples.
5. Once placement is complete, a message displays indicating that the index pattern has been applied successfully. Select OK.
6. On Add Reagents screen, reagents are already placed. Select Record Details.

7. On exit from the Add Reagents screen, the index placement is validated. Once validation is complete, a message displays indicating that the index pattern has been validated on all samples successfully. Select OK.
8. On the Record Details screen, under Reagent Lot Tracking, enter the lots for the following fields:
   - Index Primers
   - NaOH
   - PCR Master Mix
   - PCR Polymerase
9. Select Next Steps.
10. Select PCR Clean-Up (CF 139-Variant Assay) 1.1 as the next step. Select Apply.
11. Select Finish Step.

Step 5: PCR Clean-Up (CF 139-Variant Assay) 1.1

1. Add samples to Ice Bucket.
2. Select Begin Work.
3. On the Placement screen, select all samples and place in container. Select Record Details.
4. On the Record Details screen, under Reagent Lot Tracking, select the reagent lots for the following:
   - Elution Buffer
   - EtOH
   - PCR Clean-up Beads
5. Select Next Steps.
6. Select Mark protocol as complete as the next step. Select Apply.
7. Select Finish Step.

SBS MiSeqDx v1.9 Protocols

The following sections describe the key features of the Illumina Sequencing by Synthesis (SBS) MiSeqDx v1.9 protocols. These sections also describe the user interaction involved at these points in the workflow.

- Library normalization CSV file generation
- Reagent cartridge name validation
- Sample sheet generation
- Primary analysis (sequencing) results parsing, which includes generation of the run report
- Secondary analysis results parsing

Library Normalization File Generation

In each of the Illumina SBS MiSeqDx protocols, the Library Normalization (MiSeqDx) v1.1 step includes automated calculation of normalization buffer volumes. The results are provided in an autogenerated comma-separated file that is attached to the step.

How it Works

Buffer volumes are calculated by the normalizationBufferVolumes script. The script is invoked by the Create Normalization CSV automation when you select a button on the Record Details screen.

For details on the normalizationBufferVolumes script, see *Normalization Buffer Volumes* on page 2577.

User Interaction

1. On the Record Details screen of the Library Normalization (MiSeqDx) v1.1 step, enter values in for the following items:
    - Library volume (µl) transferred to destination plate
    - Normalized conc (nM)
    - Maximum destination container volume (µl)
    - Any other required fields
2. Select Create Normalization CSV to start the automation.
   When the normalizationBufferVolumes script has completed, the Normalization buffer volumes CSV file is generated and attached to the step. This file is in the Files area of the Record Details screen.

Reagent Cartridge Name Validation

In each of the SBS MiSeqDx and Validation protocols, the Denature, Dilute and Load Sample 1.1 step includes automated validation of the MiSeqDx reagent cartridge name.

How it Works

Automatically initiated on exit from the Placement screen of the step, the Validate Container Name automation validates the reagent cartridge name to ensure:

- The reagent cartridge Container Name has been renamed and is not the same as its Clarity LIMS ID.

```
if (output.container.name == output.container.node.@limsid) { fail(::The
Container Name cannot be the same as its LIMS ID. Please scan the Reagent
Cartridge RFID.::) }
```

- The Container Name contains only letters and numbers, does not have special characters, and is in the format `AB#######-XYZ` (eg, `RG1234567-ABC`).

```
else if (!output.container.name.matches(::^[a-zA-Z0-9-]*$::)) { fail(::The
Container Name can contain letters, numbers and dashes only. No other special
characters are allowed. Please scan the Reagent Cartridge RFID.::) };
```

- Sets the Reagent Cartridge Barcode field attached to the output that is from the sample input.

```
output.::Reagent Cartridge Barcode:: = output.container.name;
```

- Pads the suffix (the characters after the dash) of the Container Name so that it is at least five characters long. The Container Name is used by the MiSeqDx Sequencing Service to match the run data to the Clarity LIMS step in the MiSeqDx Run (MiSeqDx) step.

```
def match = output.container.name =~ /([a-zA-Z0-9]+-)([a-zA-Z0-9]{0,5})$/;
if (match.matches()) {
int barcodeSuffixLength = 5;
int zerosToAdd = barcodeSuffixLength - match.group(2).size();
output.container.::name:: = match.group(1).toString() + ::0::*zerosToAdd +
match.group(2).toString()
}
```

User Interaction

1. On the Placement screen of the Denature, Dilute and Load Sample 1.1 step, set up the reagent cartridge as follows.
   a. Using an RFID scanner, scan the RFID of the MiSeqDx reagent cartridge into the Container Name field.
   b. Place the pool of samples into the reagent cartridge.
2. Select Record Details.

On exit from the Placement screen, the automation is automatically triggered and validates the Container Name.

Sample Sheet Generation

In each Illumina SBS MiSeqDx and Validation protocol, automated sample sheet generation is configured on the Record Details screen of the Denature, Dilute and Load Sample 1.1 step. This is when the samples are loaded onto the container that will be placed in the instrument.

The integration allows for generation of a sample sheet designed to be used with the MOS (Diagnostic mode) instrument software.

By default, one MiSeqDx instrument sample sheet is created for the reagent cartridge loaded during the step. The placeholders and sample sheet files in the following table are generated by the Denature, Dilute and Load Sample 1.1 step.

Generated Placeholders and Sample Sheet Files

Item	Description
Lab Tracking Form placeholder	This placeholder allows you to manually attach a lab-specific tracking form to the step.

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Item	Description
MiSeqDx Sample Sheet file	• This CSV file is automatically generated by the LIMS for use with the MOS or MCS software. • May be opened as a text file, or as an MS Excel spreadsheet.
MiSeqDx Sample Sheet Generation Log	Automatically generated by Clarity LIMS, this log file captures any errors encountered when generating the MiSeqDx sample sheet (MOS).

How it Works

The sample sheet generation automation is configured on the Record Details screen of the Denature, Dilute and Load Sample 1.1 step.

- This automation is triggered by a button on the Record Details screen.
- The automation sets the value of the Progress field on the sample to 'Library ready for sequencing'.

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -u {username} -p {password} -i {processURI:v2}
script:setUDF -f 'Progress' -t '//input/@uri->//sample/@uri' -v 'Library ready
for sequencing'
```

- The automation starts a script that generates the sample sheet.

```
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/miseqdx/miseqdx-
extensions.jar -u {username} -p {password} -i {processURI:v2} script:generate_
miseqdx_sample_sheet -c {compoundOutputFileLuid1} -e {compoundOutputFileLuid2} -
-useSampleLimsID true --isMiSeqDx true
```

> To maintain unique values in the Sample_Name column of the sample sheet, the sample sheet generation automation script has the useSampleLimsID parameter set to true. The appendLimsID parameter must not be set to true.
>
> For the automation to display the correct instrument name when it is completed, the --isMiSeqDx parameter must be set to true.

- The automation labels the derived samples that do not have reagent labels. The label value applied is NoIndex.

```
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -u {username} -p {password} -i {processURI:v2}
script: labelNonLabeledOutputs -l 'NoIndex'
```

User Interaction

See *MiSeqDx Integration v1.9 Validation and Troubleshooting* on page 2270.

Supporting Documentation

For more information on sample sheet generation, including details on file format and contents, master step and global fields, script parameters, and configuration options, see the following documentation:

- *Illumina MiSeqDx 1.x Sample Sheet Generation* on page 2275—For details on the Diagnostic mode sample sheet designed for use with MOS software.
- *Sample Sheet Generation* on page 2045—For details on the Research mode sample sheet designed for use with MCS software.

Primary Analysis (Sequencing) Results Parsing

The MiSeqDx Run (MiSeqDx) step records information for each lane of the flow cell and generates a report summarizing the results. In addition, run parameters, run info, and a link to the run folder are automatically captured.

Generated and Captured Files

The following table describes the run information files, reports, placeholders, and links that Clarity LIMS automatically generates or captures during a sequencing run.

Table 479 Run Information Generated or Captured by MiSeqDx Run (MiSeqDx) v1.1 Step

Item	Description
Run Info Run Parameters	These XML files are automatically captured by Clarity LIMS from the instrument run folder. They include the key run parameters, many of which are parsed out into key master step and global fields.
Link to Run Folder	Automatically generated by Clarity LIMS, this is a link to the network run folder where the data that was captured from the instrument during the run is stored.

Item	Description
Illumina Run Report	• Automatically generated by Clarity LIMS, this report provides key information about the run and the samples on the flow cell. • Information includes the flow cell ID, run directory location, instrument name, as well as primary analysis metrics for the instrument – summarized per flow cell lane for the entire run, and individual reads in the case of paired-end runs. • These metrics are compared against the instrument's per lane averages, calculated using metrics from the last five sequencing runs. Any values outside of one standard deviation are highlighted. • The run report calculations assume that the flow cell has 8 lanes. If you are using a two-lane rapid-run flow cell, the calculated values will be incorrect.
Lab Tracking Form placeholder	This placeholder allows you to manually attach a lab-specific tracking form to the step.

Metadata

- Finish Date* – run completion date
- Run Type – Diagnostic or Research mode
- Status – current status of the sequencing run on the instrument
- Flow Cell ID
- Flow Cell Version
- Experiment Name – entered in software
- Read 1 Cycles
- Index 1 Read Cycles – intended Index cycles
- Index 2 Read Cycles – intended Index cycles
- Read 2 Cycles
- Output Folder – run folder root
- Run ID – the unique run ID
- Reagent Cartridge ID

- Reagent Cartridge Part #
- PR2 Bottle ID
- Chemistry
- Workflow

There are two additional master step fields in this step:

- Comments - field used for any comments that are attached to the run
- Report Status - hidden read-only field that tracks if the run report has been successfully uploaded

Date Run is populated with the date that the Begin Run event file is first picked up and associated with a step in Clarity LIMS, not the date of the Begin Run event itself (the date the run was performed on the instrument). This property is provided by default in Clarity LIMS and is hidden. It is used in the AUTOMATED - Run Report Generation automation.

Finish Date is populated as follows.

- If the End Run event contains a date in the format YYYY-MM-DD, Finish Date will be set to the date in the event file.
- If the End Run event does not contain a date or the date is in the wrong format, Finish Date will be set to the date when the event file is processed.

Primary Analysis Metrics

The following table lists the Real-Time Analysis (RTA) primary analysis metrics Clarity LIMS automatically captures and records, per read, for samples in each flow cell lane. These metrics are captured upon run completion.

Table 480 RTA Primary Analysis Metrics Captured by MiSeqDx Run (MiSeqDx) Step

RTA Primary Analysis Metrics	Corresponding Fields
Raw Yield (Gb)	Yield PF (Gb) R1 Yield PF (Gb) R2
% Bases >Q30	% Bases >=Q30 R1 % Bases >=Q30 R2
Cluster Density (K/mm^2)	Cluster Density (K/mm^2) R1 Cluster Density (K/mm^2) R2
Clusters Raw	Clusters Raw R1 Clusters Raw R2
Clusters PF	Clusters PF R1 Clusters PF R2

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RTA Primary Analysis Metrics	Corresponding Fields
%PF	%PF R1 %PF R2
Intensity Cycle 1	Intensity Cycle 1 R1 Intensity Cycle 1 R2
% Intensity Cycle 20	% Intensity Cycle 20 R1 % Intensity Cycle 20 R2
% Phasing	% Phasing R1 % Phasing R2
% Prephasing	% Prephasing R1 % Prephasing R2
% Aligned	% Aligned R1 % Aligned R2
% Error Rate	% Error Rate R1 % Error Rate R2

How it Works

The sequencing service may run on Clarity LIMS server in an on-premise environment or on a remote server in a SaaS environment. The service detects event files that the instrument software (RTA) is producing as the run progresses. These event files let the service know where to find the run data.

As the run data are written out and the events come in (Begin Run, Cycle Complete, End Run), the data are matched to the step based on the reagent cartridge ID. This value was entered as the Container Name on the Denature, Dilute and Load Sample 1.1 step. The read-only field values on the Record Details screen are populated accordingly.

Run report generation works as follows.

- Run report generation is asynchronous. The sequencing service does not wait for the report generation to complete before processing the next event file.
- The report is generated by the AUTOMATED - Run Report Generation automation, which is included in the BaseSpaceLIMS-miseqdx-extensions package.
- The AUTOMATED - Run Report Generation automation is configured on the MiSeqDx Run (MiSeqDx) 1.1 step. The default command line is as follows:

```
bash -c "/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/miseqdx/miseqdx-sequencing-report.jar -i
{processLuid} -r {compoundOutputFileLuid0}_RunReport.pdf -l
{compoundOutputFileLuid1}_RunReportLogFile.html
```

- The automation is configured to be invoked by a button on the Record Details screen. However, it is automatically started by the sequencing service when it has finished processing the end run event.

  It is not intended for the user to select the AUTOMATED - Run Report Generation button. The run report is generated and attached to the step automatically.

- An additional shared result-file placeholder is configured on the step. This placeholder is for a new log file—*Illumina Run Report Log File*—that is generated and attached to the step when the report runs.
- The run report can be successfully generated in a hosted deployment of Clarity LIMS where the sequencing service is running on a remote server on the customer network.
- The automation also sets the Report Status field to Completed.

```
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
script:evaluateDynamicExpression -exp 'step.::Report Status:: = ::Completed::' -
t 'false' -log tempLog.html"
```

Following the sequencing run, the run report is automatically generated by the AUTOMATED - Run Report Generation automation. Do not change this name. This name is expected by the sequencing service that captures instrument run results. The base name is stored in the sequenceProcessBaseName database property. If this name is changed without the database property being updated, the 'flow cell ID <-> sequencing step base name' matching will fail.

The Verify Report Status automation prevents movement to the Assign Next Steps screen unless the Report Status field is set to Completed (this is done by the previous automation after the run report is attached to the step).

```
bash -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
script:evaluateDynamicExpression -exp 'if (!step.hasValue( ::Report Status:: )
|| step.::Report Status:: != ::Completed::) { fail(::Cannot advance because
expected files are missing, please wait until the RunReport, RunInfo and
RunParameters files are generated and attached before completing the step.::) }
' -t 'false' -log tempLog.html"
```

User Interaction

On the Record Details screen, do not select the AUTOMATED - Run Report Generation button. The run report is generated and attached to the step automatically. This process may take a while.

On the Record Details screen of the MiSeqDx Run (MiSeqDx) 1.1 step:

1. Run the instrument. The read-only field values are automatically populated as the instrument runs.
2. After the run has completed, observe the Record Details screen of the MiSeqDx Run (MiSeqDx) 1.1 step for the following.
    - The autopopulated read-only fields.
    - The generated and attached Illumina Run Report and Log File.
    - The attached Run Parameters and Run Info files.
    - The generated and attached Link to Run Folder.
    - QC flags set on each lane.
3. [Optional] In the Files section, upload the Lab Tracking Form.
4. Select Next Steps.
   The Verify Report Status automation should run and allow you to go to the Assign Next Steps screen.
5. On the Assign Next Steps screen, assign the samples to the Variant Calling (MiSeqDx) 1.1 step.
6. Select Finish Step.

Secondary Analysis Output Files Attachment

The Variant Calling (MiSeqDx) 1.1 step attaches secondary analysis output files for each of the samples.

In addition, a Combined Variant Call File, a text file, and a variant call file for each individual sample are automatically captured following the completion of secondary analysis.

How it Works

Following primary analysis, secondary analysis runs on the MiSeqDx instrument. Clarity LIMS detects the completion of secondary analysis and lets the service know where to find the result files. While the EndRun event is processed successfully for primary analysis, the sequence service generates a BeginSecondaryAnalysis event file in the same directory with the `miseqdex.seqservice.eventFileDirectory` property.

An example of how Clarity LIMS shows the location of the secondary analysis event file is:

```
netFolder = D:\Illumina\MiSeqOutput\161017_M99999_0047_FC1234567-
ABCDErunNetworkLocation = /mnt/ussd-dev-mdx01/MiSeqOutput/161017_M99999_0047_
FC1234567-ABCDEeventType = BeginSecondaryAnalysissoftwareType = MRScreationTime
= Thu Oct 20 22:56:32 GMT 2016
```

This event file signals that secondary analysis has started. The sequence service periodically checks if the analysis is complete by looking for the `CompletedJobInfo.xml` file. This file is in the folder specified by the runNetworkLocation parameter of the event file.

The frequency of checking is defined by the `miseqdx.seqservice.synchronizationPeriod` property.

If the completion file appears, the sequence service processes the event. The event is archived in a similar way to how the EndRun event file is handled. If the event completion does not appear within the number days specified by the `miseqdex.seqservice.ignoreUnmatchedContainerIdsWaitDays` property, the service stops monitoring the event file and the file is archived.

Captured Files

The following table describes the Variant Call Format (VCF) files that Clarity LIMS captures and attaches to the step after the secondary analysis.

Table 481 Variant Call Format Files Captured by the LIMS After Secondary Analysis

File Type	Description
Combined Variant Call File	Variant call VCF files for all samples are compressed into a zip file and attached to the step for every run. Available for all 3 assays.
Combined Output Text File	For CF Clinical Sequencing Assay and CF 139-Variant Assay only, a text file containing the summary of variant call information for all samples is attached for every run.
Variant Call File per sample	The general variant call VCF file (not CFTR VCF file) for each sample is attached. Available for all 3 assays.

User Interaction

On the Record Details screen of the Variant Calling (MiSeqDx) 1.1 step:

1. Make sure that the VCF has the correct name based on the sample sheet.
   The file-naming format for the VCF file is SampleName_S#.vcf. In this format, the SampleName is the value in the Sample_Name column of the sample sheet, and # is the sample number determined by the order in which samples are listed in the sample sheet.
2. After secondary analysis has completed, the following files are attached to the step:
   - Combined Variant Call File—a compressed VCF file (zip file) containing all the variant call files, including the CFTR specific VCF files.
   - Combined Output Text File—a combined text file containing a summary of all the sample metrics.
   - Variant Call File per individual sample
3. QC flags are set on each lane.

Installed Components

The Illumina MiSeqDx Integration RPM package installs the following components.

This integration requires installation of the associated NGS Extensions Package (see Release Notes).

Scripts and Files

Files Installed	Location and Description
configure_extensions_ miseqdx_workflow.sh	/opt/gls/clarity/config/ Script that imports the integration configuration.
miseqdx-extensions.jar	/opt/gls/clarity/extensions/miseqdx Jar file containing API-based LIMS extensions used throughout the protocols.
configure_extensions_ miseqdx_ sequencingservice.sh	/opt/gls/clarity/config/extensions/miseqdx Script that imports the database properties for the integration.
log4j2.xml	/opt/gls/clarity/extensions/miseqdx/SequencingService/conf File containing the settings for the sequencing jar logging.
miseqdx-sequencing-report.jar	/opt/gls/clarity/extensions/miseqdx/ Jar file containing API-based LIMS extensions used for report generation.
miseqdx-sequencing.jar	/opt/gls/clarity/extensions/miseqdx/SequencingService Sequencing service jar file that captures the run results.
miseqdx_seqservice	/opt/gls/clarity/extensions/miseqdx/SequencingService/bin Provides commands for interacting with the sequencing service. Accessible at `/etc/init.d`
run_miseqdx_seqservice	/opt/gls/clarity/extensions/miseqdx/SequencingService/bin Called by `miseqdx_seqservice-v1`, this script performs some basic system validation, then starts the sequencing service.
MiSeqDxIntegrator.log	/opt/gls/clarity/extensions/miseqdx/SequencingService Log file for Sequencing service.

Database Properties

The following table lists the database properties installed with the Illumina MiSeqDx Integration Package. The following constraints are present when using the database properties:

- Sequencing runs are matched using the flow cell ID and the base name of the sequencing step – MiSeqDx Run (MiSeqDx).

- Do not change this name – it is expected by the sequencing service that captures instrument run results. The base name is stored in the sequenceProcessBaseName database property shown in Table 2. If this name is changed without the database property being updated, the 'flow cell ID <-> sequencing step base name' matching system will fail.
- If necessary, you may modify the step name by editing or adding to the text after the base name portion. This part of the text is not used in the matching system. For example, you could change MiSeqDx Run (MiSeqDx) 1.1 to MiSeqDx Run (MiSeqDx) v1.

Several additional properties, each with the '99' suffix appended to their name, are also installed. These properties are intended for use by the Clarity LIMS support team in automated validation tests and are not listed in the table.

Table 482 Database Properties Installed with the Illumina MiSeqDx Integration Package

Property	Description	Default Value
miseqdx.seqservice.ignoreUnmatchedContainerIds	A flag indicating if event files that cannot be matched to flow cells in Clarity LIMS should be archived after a period of time (true), or continually reprocessed (false).	false To prevent the `gls_events` file directory from becoming cluttered, it is recommended that the value of this property is set to 'true'.
miseqdx.seqservice.ignoreUnmatchedContainerIdsWaitDays	The number of days between when the event is created and the event file is archived.	14
miseqdx.seqservice.synchronizationPeriod	Invocation period in seconds.	60

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Property	Description	Default Value
miseqdx.seqservice.sequenceProcessBaseName	Sequencing process type base display name. Partial matching is used to look up the process type.	MiSeqDx Run (MiSeqDx)
miseqdx.seqservice.eventFileDirectory.1	A network location monitored for event files.	/mnt/gls_events
miseqdx.seqservice.netPathPrefixSearch.1*	The network directory prefix contained in the event file.	\\nas\network\run_data
miseqdx.seqservice.netPathPrefixReplace.1	The mapped network directory mount name on the server used to access the run data directory.	/mnt/run_data
miseqdx.seqservice.eventFileDirectorySuffixes	A list of eventFileDirectory path entries to monitor for event files. The value is one or more comma-separated integers.	99

Property	Description	Default Value
miseqdx.seqservice.runReportViewsVersion	The current version of the Run Report views in the database. The value 0 represents the state before the views are created. This property is automatically updated by the run report.	0
miseqdx.seqservice.netPathPrefixSearchReplaceSuffixes	A list of netPathPrefix search and replace entries for transforming Windows to Linux network paths. The value is one or more comma-separated integers.	99
miseqdx.seqservice.variantCallingProcessBaseName 'Variant Calling (MiSeqDx)'	Sequencing process type base display name. Partial matching is used to look up the process type.	Variant Calling (MiSeqDx)

*It is possible to configure support for multiple, identical seqservice.netPathPrefixSearch property values. For details, see *Configure Multiple Identical netPathPrefixSearch Values* on page 2596.

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Reagent Categories/Label Groups

The following reagent categories/label groups are included in the default configuration for the MiSeqDx Integration Package:

- CF 139-Variant Assay Indexes
- CF 139-Variant Assay 8-Sample Indexes
- CF Clinical Sequencing Assay 8-Sample Indexes
- Universal Kit Indexes
- Universal Kit 8-Sample Indexes

Reagent Kits

The following reagent kits are included in the default configuration for the Illumina MiSeqDx Integration Package:

- CF 139-Variant Assay-Oligo Pool
- CF Clinical Sequencing Assay-Oligo Pool
- Custom Oligo Pool
- Elution Buffer
- EtOH
- Extension-Ligation Mix
- Hybridization Buffer
- Index Primers
- Library Beads
- Library Dilution Buffer
- Library Normalization Diluent
- Library Normalization Wash
- Library Storage Buffer
- MiSeqDx Flow Cell - CF 139-Variant Assay
- MiSeqDx Flow Cell - CF Clinical Sequencing Assay
- MiSeqDx Flow Cell - Universal kit
- MiSeqDx SBS Solution (PR2) - CF 139-Variant Assay
- MiSeqDx SBS Solution (PR2) - CF Clinical Sequencing Assay
- MiSeqDx SBS Solution (PR2) - Universal Kit
- NaOH
- PCR Clean-Up Beads
- PCR Master Mix
- PCR Polymerase
- Stringent Wash Buffer
- Universal Wash Buffer

Controls

The following controls are included in the default configuration for the MiSeqDx Integration Package:

- Negative Control for MiSeqDx
- Positive Control for MiSeqDx

Container Types

All one-dimensional container types with both numeric rows and numeric columns are supported.

The following container types are included in the default configuration for the MiSeqDx Integration Package:

- MiSeqDx Flow Cell - Universal Kit

- MiSeqDx Flow Cell - CF 139-Variant Assay
- MiSeqDx Flow Cell - CF Clinical Sequencing Assay
- MiSeqDx Reagent Cartridge - CF 139-Variant Assay
- MiSeqDx Reagent Cartridge - CF Clinical Sequencing Assay
- MiSeqDx Reagent Cartridge - Universal Kit

Instrument Integration

The following information provides an overview of the steps performed by the Clarity LIMS support team when configuring the instrument for use with the MiSeqDx Integration to Clarity LIMS.

Configure the MiSeqDx as follows.

1. Create a directory on the local computer to hold the batch files. These batch files write event files to the network-attached storage (NAS) shares.
2. Create a directory on the NAS to hold the event files.
3. Modify the software configuration files to call the batch files that create the event files.
4. Update sequencing service default database properties to match the specifics of the installation.

> To make sure that your Illumina instrument warranty remains valid, the instrument integration must be performed and maintained by the Clarity LIMS support team. To perform this integration, the Clarity LIMS support team will require direct access to the instrument via WebEx or Remote Desktop while the instrument is idle.

Configuring Instrument Names

We recommend configuring instrument names in Clarity LIMS to track the specific lab instrument on which a run was completed.

This information should be configured on the MiSeqDx Run (MiSeqDx) 1.1 step, and should be set to the same name as is configured in the Illumina sequencing software (ie, this will be value of the Instrument Run ID field described in the *Metadata* on page 2258 section).

When the integration reads the Instrument Run ID field in the run results, it will update the instrument on the step in Clarity LIMS. This update eliminates the need to enter this information manually.

For instructions on configuring instrument names, refer to the Clarity LIMS Core Functionality documentation.

Configuring Sample Sheet Generation

Sample sheet generation occurs on the step prior to the sequencing run—Denature, Dilute and Load Sample 1.1—which is the step where samples are placed on the container that will be loaded in the instrument.

By default, an instrument sample sheet is created for the container loaded during the Denature, Dilute and Load Sample 1.1 step.

Rules and Constraints

This integration operates with the following constraints:

- The Reagent Cartridge ID must be unique. There should not be multiple containers in the system with the same name.
- The reagent cartridge ID must be scanned as the Container Name on the Denature, Dilute and Load Sample 1.1 step.
- For MOS sample sheet generation constraints, see *Illumina MiSeqDx 1.x Sample Sheet Generation* on page 2275.

MiSeqDx Integration v1.9 Validation and Troubleshooting

This section explains how to use the MiSeqDx Validation workflow that is included in the Illumina MiSeqDx Integration Package. The workflow allows for simple validation of the following functionality:

- Auto-placement of reagent indexes on the PCR Amplification step
- Generation of a sample sheet file for use with the MiSeqDx Operating Software (MOS) on the Denature, Dilute and Load Sample step

In addition, this section provides tips to help you troubleshoot the system.

All validation steps assume that you have installed the MiSeqDx Integration Package and have imported the default Clarity LIMS configuration. For compatibility information, see the *Release Notes*. Clarity LIMS v4.x is no longer supported in MiSeqDx Integration Package v1.9 or in subsequent releases.

Instructions for configuring sample sheet generation are provided in *MiSeqDx Integration v1.9 Configuration and User Interaction* on page 2245

Validate Auto-Placement of Indexes and Sample Sheet Generation

After installing and configuring the integration package, and configuring sample sheet generation (see *v1.9 Configuration and User Interaction*), follow the steps to activate the MiSeqDx Validation workflow and run samples through it. The autoplacement of indexes validation occurs in Run PCR Amplification step and sample sheet generation validation occurs in Run Denature, Dilute and Load Samples step.

Activate Workflow, Add and Assign Samples

1. In the Clarity LIMS web interface, on the Configuration > Workflows screen, activate one of the three Validation workflows:
    - MiSeqDx Validation (CF 139-Variant Assay) 1.1

- MiSeqDx Validation (CF Clinical Sequencing Assay) 1.1
- MiSeqDx Validation (Universal Kit) 1.1

2. On the Projects and Samples screen, create a project and assign samples as follows.
   a. Create a test project and add samples to it.
      The project must contain between 9 and 96 samples.
   b. Assign your samples to the MiSeqDx Validation workflow.

Run Extension-Ligation of Bound Oligos Step

1. In Lab View, locate the MiSeqDx Validation protocol. You will see your samples queued for the Extension-Ligation of Bound Oligos step.
   Prepare the samples as follows.
2. Add the samples to the Ice Bucket.
3. On the Placement screen, place the samples into the output container.
4. Select Record Details.
5. On the Record Details screen, under Reagent Lot Tracking, select the Extension-Ligation Mix reagent lot used in the step.
   You may need to activate the lot on the Reagents / Reagents and Controls screen.
6. Select Next Steps.
7. On the Assign Next Steps screen, assign the samples to the PCR Amplification step.
8. Select Finish Step.

Run PCR Amplification Step

1. In Lab View, locate the MiSeqDx Validation protocol. You will see your samples queued for the PCR Amplification step.
2. Add the samples to the Ice Bucket.
3. In the Reagent Label Options panel, in the Group of labels list, select CF 139-Variant Assay Indexes (or the equivalent for the workflow you activated).
4. Select Begin Work.
5. On the Placement screen, place the samples into the output container.
6. Select Add Reagents.
   On entry to the Add Reagents screen, the Auto Place Indexes automation is automatically invoked and the reagents are placed on the samples.
7. After placement is complete, a message displays indicating that the index pattern has been applied successfully. Select OK.
8. On the Add Reagents screen, reagents are already placed. Select Record Details.

On exit from the Add Reagents screen, the Validate Index Placement automation is automatically invoked and index placement is validated.

9. When validation is complete, a message displays indicating that the index pattern has been validated on all samples successfully. Select OK.
10. On the Record Details screen, under Reagent Lot Tracking, select the lots for the following items:
    - Index Primers
    - NaOH
    - PCR Master Mix
    - PCR Polymerase
11. Select Next Steps.
12. On the Assign Next Steps screen, assign samples to the Library Pooling (MiSeqDx) 1.1 step.
13. Select Finish Step.

Run Library Pooling (MiSeqDx) 1.1 Step

1. Return to Lab View and locate the MiSeqDx Validation protocol. You will see your samples queued for the Library Pooling (MiSeqDx) 1.1 step.
   Prepare the samples as follows.
2. Add samples to the Ice Bucket.
3. In the Add Control Samples panel, select PhiX Internal Control. Select Begin Work.
   a. On the Pool Samples screen, create a pool of samples. Select Place Samples.
   b. On the Placement screen, place the pool into the output container. Select Record Details.
   c. Under Reagent Lot Tracking, select the reagent lot used in the step.
   d. Under Step Details, enter the concentration value in the Normalized conc. (nM) field.
4. Select Next Steps.
5. On the Assign Next Steps screen, assign the pool to the Denature, Dilute and Load Samples step.

Run Denature, Dilute and Load Samples Step

1. Return to Lab View and locate the MiSeqDx Validation protocol.
   You will see your pooled samples queued for the Denature, Dilute and Load Sample step.
2. Place the pooled samples as follows.
   a. Add the pool to the Ice Bucket.
   b. On the Placement screen, place the pool into a MiSeqDx reagent cartridge.
   c. Select Record Details.
      On entry to the Record Details screen, a script validates the Container Name and prompts you to scan the reagent cartridge barcode.

3. On the Record Details screen of the Denature, Dilute and Load Sample step, generate the sample sheet as follows.

All read-only files are autopopulated.

   a. Enter the folder path for the Reference Genomes that will be used for secondary analysis in the GenomeFolder field.
   a. Under Reagent Lot Tracking, select the reagent lots used in the step.
   b. Enter the lots for MiSeqDx Flow Cell - CF 139-Variant Assay and MiSeqDx SBS Solution (PR2) - CF 139-Variant Assay.
   c. In the Step Details section, review the step parameters.
   d. Select Generate MiSeqDx Sample Sheet to generate the MiSeqDx sample sheet.
   e. Clarity LIMS attaches the generated sample sheet and log file to placeholders in the Files area of the Record Details screen. Download the files and validate their format and content.
   f. [Optional] In the Files section, upload the Lab Tracking Form to the appropriate placeholder.
4. Select Next Steps.
5. On the Assign Next Steps screen, assign the samples to the MiSeqDx Run (MiSeqDx) 1.1 step.

Validate Creation of Event Files

Validating the creation of the event files confirms the following:

- Your DESTINATION_PATH is correctly configured.
- The instrument computer can access and write to the DESTINATION_PATH.
- There are no syntax errors in the Clarity LIMS batch file.

Follow the steps below to confirm that event files are created by the batch file in the destination path. The steps assume that the default configuration has been successfully imported.

1. In `C:\gls`, double-click `gls_event_mos_rta.bat`.
2. Confirm that an empty event file appears in the configured DESTINATION_PATH.

Troubleshooting

If an automation trigger does not appear to run its corresponding scripts, see:

- *Troubleshooting Automated Informatics* in the LIMS documentation in the Automated Informatics section.
- *Troubleshooting EPP / Automation* in the API > Overview > API Advanced Topics section.

If an error occurs that does not provide direction on how to proceed, troubleshoot the error as follows.

1. Confirm the version of the installed Illumina MiSeqDx Integration Package by running the `rpm -qa | grep -i miseqdx` command from the server console.
2. If the error is related to retrieving run results data, review the MiseqDxIntegrator.log.

3. Contact the Clarity LIMS Support team, supplying the relevant information from the troubleshooting already performed.

MiSeqDx Integration v1.9 Manual Upgrade

MiSeqDx Integration v1.9 includes the MiSeqDx v1.1 workflow upgrade. This upgrade is typically done through the RPM. To upgrade the workflow manually, update the Generate MiSeqDx Sample Sheet automations used in the following master steps:

- Denature, Dilute and Load Sample (Universal Kit) 1.0
- Denature, Dilute and Load Sample (CF Clinical Sequencing Assay) 1.0
- Denature, Dilute and Load Sample (CF 139-Variant Assay) 1.0

If these master steps do not share the same automation command line, update the individual automation command lines for each master step.

Before updating the automations, make sure the MiSeqDx v1.0 workflow is installed.

Update Generate MiSeqDx Sample Sheet Automation

1. In Clarity LIMS, under Configuration, select the Automation tab.
2. Under the Step Automation tab, select the Generate MiSeqDx Sample Sheet automation.
3. In the Command Line field, add an extra hyphen before `-useSampleLimsID` and `-isMiSeqDx true` as follows.
   Changes to the existing command line are in red.

```
bash -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -u {username} -p {password} -i {processURI:v2}
script:setUDF -f 'Progress' -t '//input/@uri->//sample/@uri' -v 'Library ready
for sequencing' && /opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/miseqdx/miseqdx-extensions.jar -u {username} -p
{password} -i {processURI:v2} script:generate_miseqdx_sample_sheet -c
{compoundOutputFileLuid1} -e {compoundOutputFileLuid2} --useSampleLimsID true --
isMiSeqDx true && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -u {username} -p {password} -i {processURI:v2}
script:labelNonLabeledOutputs -l 'NoIndex'"
```

MiSeqDx Integration v1.9 Release Notes

These Release Notes describe the key changes to software components for the Clarity LIMS MiSeq Integration Package version 1.9. This is an optional software update.

Compatibility

This version of the integration package is compatible with the following software:

- BaseSpace Clarity LIMS v5.1 or later

- NGS Extensions Package v5.21 or later

New Features

Features provided with this release of the integration package are as follows.

- Support for Clarity LIMS v6.0. Clarity LIMS v4.x and v5.0 are not supported.
- Tech refresh to upgrade third party libraries.
- Fixes to address the following Apache log4j vulnerabilities:
  - CVE-2021-44228
  - CVE-2021-45046
  - CVE-2021-45105
  - CVE-2021-44832

Defects Fixed

Fixed MiSeqDx run report to display full footer.

Known issues

None

Illumina MiSeqDx 1.x Sample Sheet Generation

Package Version: BaseSpace Clarity LIMS MiSeqDx

Overview

The Illumina MiSeqDx Integration Package allows for automatic generation of a sample sheet to be used with the MiSeqDx instrument software. The format of this sample sheet is designed for the instrument when it is running in Diagnostic mode.

Note:

- If you are running the MiSeqDx instrument in Research Use Only (RUO) mode, seeMiSeq Sample Sheet Generation.
- MiSeqDx does not support bcl2fastqv2 sample sheet generation.

How sample sheet generation works

Sample sheet generation is configured on the step prior to the sequencing run – Denature, Dilute and Load Sample, which is the step where samples are placed on the flow cells or reagent cartridges that will be placed in the instrument.

The sample sheet is generated by means of a script, which the lab user initiates by clicking a button on the Record Details screen of the step. This generates a sample sheet file for the container loaded during the step, where the name of the sample sheet will be <reagent cartridge barcode ID>.csv. The user then downloads the sample sheet from the LIMS and uploads it to the instrument software.

MiSeqDx assays support

The following assays are supported by the MiSeqDx sample sheet generation script:

- CF 139-Variant Assay
- CF Clinical Sequencing Assay
- Universal Kit

Configured master step fields/step UDFs

Sample sheet format is controlled via master step field / step user defined field (UDF) configuration, where key step fields are pre-populated with values specific to each protocol step. These values are not editable, and their configuration should not be modified.

For details, see Sample sheet data and File format and contents sections below.

The fields listed in the following table are available on the Denature, Dilute and Load Sample step and will be placed into the sample sheet.

Field Name	*Field Type*	*Required?*	*Notes*
Experiment Name	Text	No	Entered by the user
Workflow	Text	Yes	Value set to Amplicon for CF 139-Variant Assay and CF Clinical Sequencing Assay Value set to Custom Amplicon for Universal Kit
Description	Text	No	Entered by the user
Assay	Text	No	Configured with the following preset values: • CF 139-Variant • CF Clinical Sequencing • MiSeqDx Universal
Amplicon Workflow Type	Text	No	Configured with the following preset values: • CF139VARIANTASSAY • CFCLINICALSEQUENCINGASSAY • MiSeqDx Universal
Application	Text	Yes	Configured with the following preset values: • CF 139-Variant Assay • CF Clinical Sequencing Assay • MiSeqDx Universal
PhiX Control added?	Check box	No	Default set to false
VariantCaller	Text	No	Value set to Starling, a legacy variant caller
Variant Min Quality Cutoff	Numeric	No	Value set to 100

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Field Name	*Field Type*	*Required?*	*Notes*
GenomeFolder	Text	Yes	Required for secondary analysis
Control CAT Manifest	Text	No	Preset value CFTRManifest.txt
Custom CAT Manifest	Text	No	Manifest file for custom assay
Read 1 Cycles	Numeric	Yes	Configured with range 0-1000. Value set to 151
Read 2 Cycles	Numeric	Yes	Configured with range 0-1000. Value set to 151

Submitted sample global fields/UDFs

Field Name	Field Type	Required?	Notes
Reference Genome	Text	No	Optionally used to populate the GenomeFolder value for individual samples in the sample sheet.
Control?	Text	No	Used to indicate a control sample that is represented as a submitted sample in the LIMS.

Script parameters and usage

Parameter	Description
u, username	LIMS username (Required)
p, password	LIMS password (Required)
i, processURI	LIMS process URI (Required) (lowercase I)
c, csvFileLimsIds	Sample sheet CSV file LIMS ID (Required - may be provided multiple times)
e, errorLogFileName	Log file name (Required)
l, useProjectLimsID	Project LIMS ID will be used instead of project name in the Project column of the sample sheet (Optional) Accepted values: true or false. Provide with quotes e.g. -l 'true' (lower case L)
s, useSampleLimsID	Should be set to true Sample LIMS ID will be used instead of sample name in the SampleName column of the sample sheet (Optional) (See Enabling unique FASTQ file names) Accepted values: true or false. Provide with quotes e.g. -s 'true'
a, appendLimsID	Should be set to false LIMS ID of the protocol step will be appended to sample names in the SampleName column of the sample sheet. (Optional) Accepted values: true or false. Provide with quotes e.g. -a 'true'

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Usage

Below is the default command line that ships with the Denature, Dilute and Load Sample (CF 139-Variant Assay) 1.0 step.

```
bash -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -u {username} -p {password} -i {processURI:v2} script:setUDF -f 'Progress' -t '//input/@uri->//sample/@uri' -v 'Library ready for sequencing' && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/miseqdx/miseqdx-extensions.jar -u {username} -p {password} -i {processURI:v2} script:generate_miseqdx_sample_sheet -c {compoundOutputFileLuid1} -e {compoundOutputFileLuid2} -useSampleLimsID true && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -u {username} -p {password} -i {processURI:v2} script:labelNonLabeledOutputs -l 'NoIndex'"
```

Support for container types

Single well container types, and all one-dimensional container types with both numeric rows and numeric columns, are supported.

Sample sheet data

The following table lists and describes the fields included in the MiSeqDx sample sheet.

Note the following:

- If no upstream pooling is detected, LIMS will populate the sample sheet with the SampleID and SampleName of the submitted sample. Other fields are populated with data from the samples that were input to the step (i.e. derived samples).
- If upstream pooling is detected, LIMS will populate the sample sheet with the first upstream pooled inputs found – not with the submitted sample or step input fields.

About controls

Control samples may be one of the following:

- Built-in BaseSpace Clarity LIMS control samples
- Submitted samples with field / UDF Control? set to true

Field Name	*Contents*	*Comments*
[Header]		Optional
WMFileVersion	Illumina Worklist Manager Version number	Optional
Date	The date the sample sheet was generated.	Optional

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Field Name	*Contents*	*Comments*
Workflow	Master step field / Step UDF of the same name.	Required
Application	Populated with CF 139-Variant Assay or Cystic Fibrosis 139 Variant Assay or MiSeqDx Universal.	Required
Assay	Master step field / Step UDF of the same name.	Optional
Description	Master step field / Step UDF of the same name.	Optional
Chemistry	Populated with Amplicon.	Required for all workflows.
[Manifests]		If a value is provided for only a single CAT manifest file, then all samples in the sample sheet will be given the designation (A) associated with that CAT type.
A	Master step field / Step UDF Control CAT Manifest. Typically a path to a file.	Optional, but must be a real path. Convention indicates this is the Control CAT. Control samples will be given the designation A in the Data section.
[Reads]	Master step fields / Step UDFs of the same name Read 1 Cycles and Read 2 Cycles.	Required. Read cycle entries are listed beneath the [Reads] heading, in the first column of the spreadsheet. One entry for single read, two entries for paired end. Index reads are determined by the MOS, based on the indexes on the inputs. Read 1 cycles is required for all workflows. Read 2 cycles is optional.
[Settings]		

Field Name	*Contents*	*Comments*
AmpliconWorkflowType	Master step field / Step UDF Amplicon Workflow Type. Populated with values CF139VARIANTASSAY or CFCLINICALSEQUENCINGASSAY or MiSeqDx Universal	Optional
VariantCaller	Master step field / Step UDF of the same name.	Optional
VariantMinimumQualCutoff	Master step field / Step UDF Variant Min Quality Cutoff.	Optional
[Data]		
Sample_ID	Populated with the LIMS ID of the sample if pooled, or the LIMS ID of the submitted sample if not pooled.	Required for all workflows.
Sample_Name	Populated with the sample name if pooled, or the submitted sample name if not pooled. If script parameter useSampleLimsID is provided on the command line, the LIMS ID of the sample will be used instead of the name. The additional -a command line option appends the LIMS ID to the end of this value, e.g. *Sample1-1234*	Always present, even if not populated. See Script parameters.
Sample_Plate	Name of the Container that the Sample resides in as recorded in the LIMS.	Always present.
Sample_Well	Container well location of the sample. If a sample is part of a pool, this will list the well location of the sample that was added to the pool.	Always present.
Sample_Project	The name of the project in the LIMS, that the sample belongs to.	Always present.

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Field Name	*Contents*	*Comments*
Control	Blank for a normal sample. Populated with value positive when Positive Control for MiSeqDx has been added to the pool. Populated with value negative when Negative Control for MiSeqDx has been added to the pool. Controls must have an index.Controls in these cases otherwise look just like the other samples.	Optional
index	Determined from the reagent label. Uses the Sequence attribute value from Index Reagents. Dual index reagents will contain a hyphen-separated DNA sequence; this field will use the first half of that value.	Required if more than one input.
I7_Index_ID	Determined from the name of the index reagent type. Dual index names will be hyphen-separated; this field will use the first half of that value.	Required if more than one input.
index2	Determined from the reagent label. Uses the Sequence attribute value from Index Reagents. Dual index reagents will contain a hyphen-separated DNA sequence; this field will use the second half of that value.	Optional
I5_Index_ID	Determined from the name of the index reagent type. Dual index names will be hyphen-separated; this field will use the second half of that value.	Optional

Field Name	*Contents*	*Comments*
Manifest	A	Required. Value determined by the entries in the Manifests section.
GenomeFolder	If master step field / step UDF Use submitted sample details for Genome Folder location is true, this is populated with the value of submitted sample global field / UDF Reference Genome." Otherwise, populated with the value of master step / step UDF GenomeFolder.	Required folder path for ReferenceGenomes used for secondary analysis.
Description		Optional

Illumina MiSeqDx Integration v1.5 Configuration and User Interaction

Compatibility: See Release Notes

Contents

The Illumina MiSeqDx Integration Package supports the integration between BaseSpace Clarity LIMS and Illumina MiSeqDx instruments.

The integration allows for automated tracking of an Illumina sequencing run in the LIMS. This includes tracking instrument run status, generating run report, capturing and parsing run statistics, and uploading variant call files to the LIMS. In addition, this integration provides automated generation of a sample sheet file for use with the MiSeq Operating Software (MOS).

- This guide describes the integration between LIMS and the IlluminaMiSeqDx system. It includes information about protocols and automations, configuration options, installed components, and rules and constraints.
- For instructions on validating and troubleshooting the Illumina MiSeqDxIntegration Package, see the Illumina MiSeqDx Integration v1.5 Validation and Troubleshooting Guide.
- For overview information about how the Illumina – LIMS interface works, and which events are monitored, see How the Illumina Instrument and LIMS Software Interact.

Overview

This Illumina MiSeqDx integration includes:

- A MiSeqDx RPM that supports Diagnostic (Dx) mode.
- A MiSeq RPM that supports Research (RUO) mode.

- Preconfigured LIMS protocols that map to library prep and sequencing lab protocols and instrument runs.
- Preconfigured protocols that allow for validation of the sample sheet generation and index placement functionality.
- Automatic generation of the following:
  - A sample sheet file for use with the MOS (Diagnostic mode) instrument software
  - A run report, which includes the current run's statistics
- Automated tracking in the LIMS of the following:
  - Progress and metrics of a MiSeqDx sequencing run
  - Per-instrument sequencing runs
  - Sequencing run parameters
  - Real Time Analysis (RTA) run directory location and other run specific information
- Ability to directly monitor run status (cycle number) across multiple instruments from within the LIMS.
- Ability to attach Variant Call Format (VCF) files to the LIMS.

Information on these features is provided in this guide.

Protocols and automations

The following out-of-the-box protocols are included in the Illumina MiSeqDx Integration Package:

- CF 139-Variant Assay Library Prep 1.0
- CF Clinical Sequencing Assay Library Prep 1.0
- Universal Kit Library Prep 1.0
- Illumina SBS MiSeqDx (CF 139-Variant Assay) 1.0
- Illumina SBS MiSeqDx (CF Clinical Sequencing Assay) 1.0
- Illumina SBS MiSeqDx (Universal Kit) 1.0

The following validation protocols are installed; each is included in a workflow of the same name:

- MiSeqDx Validation (CF 139-Variant Assay) 1.0
- MiSeqDx Validation (CF Clinical Sequencing Assay) 1.0
- MiSeqDx Validation (Universal Kit) 1.0

Figures 1 - 7 show the protocols, and the steps each includes, as displayed to the lab scientist working in the LIMS Lab View.

Note: In 'Assay' workflows, remember to include a QC validation protocol between the Library Prep and Illumina SBSprotocols. The out-of-the-box workflow does not include a QC protocol.



Figure 1: CF 139-Variant Assay Library Prep 1.0 protocol and steps

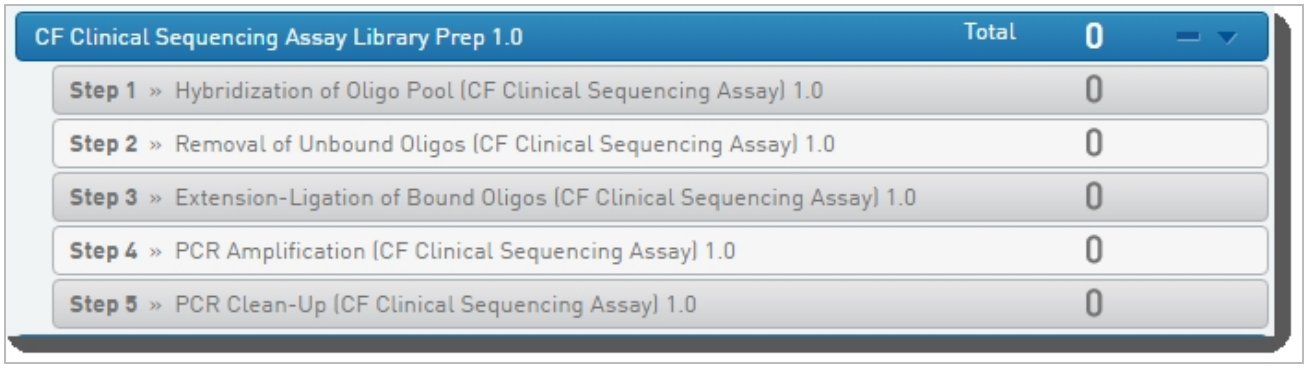


Figure 2: CF Clinical Sequencing Assay Library Prep 1.0 protocol and steps



Figure 3: Universal Kit Library Prep 1.0 protocol and steps

Figure 4: Illumina SBS MiSeqDx (CF 139-Variant Assay) 1.0 protocol and steps

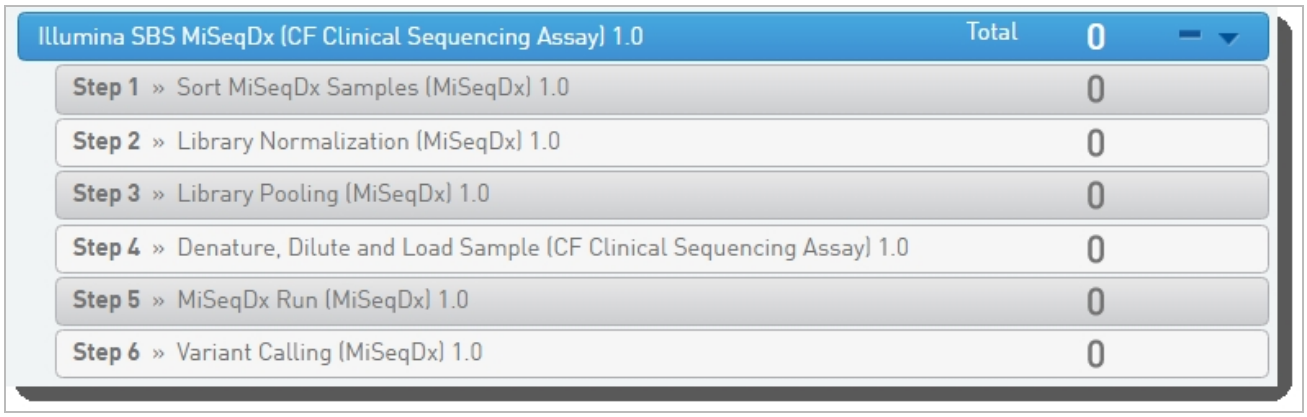


Figure 5: Illumina SBS MiSeqDx (CF Clinical Sequencing Assay) 1.0 protocol and steps

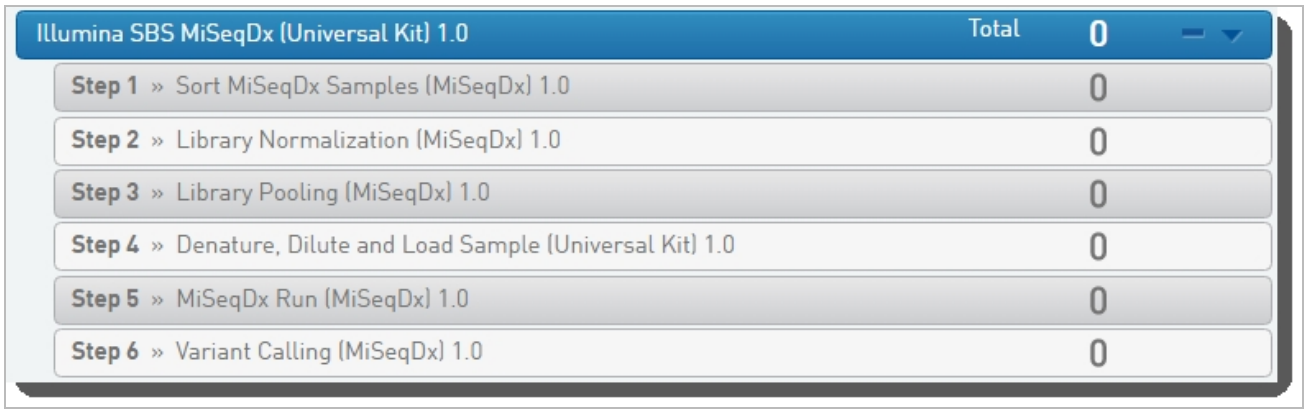


Figure 6: Illumina SBS MiSeqDx (Universal Kit) 1.0 protocol and steps

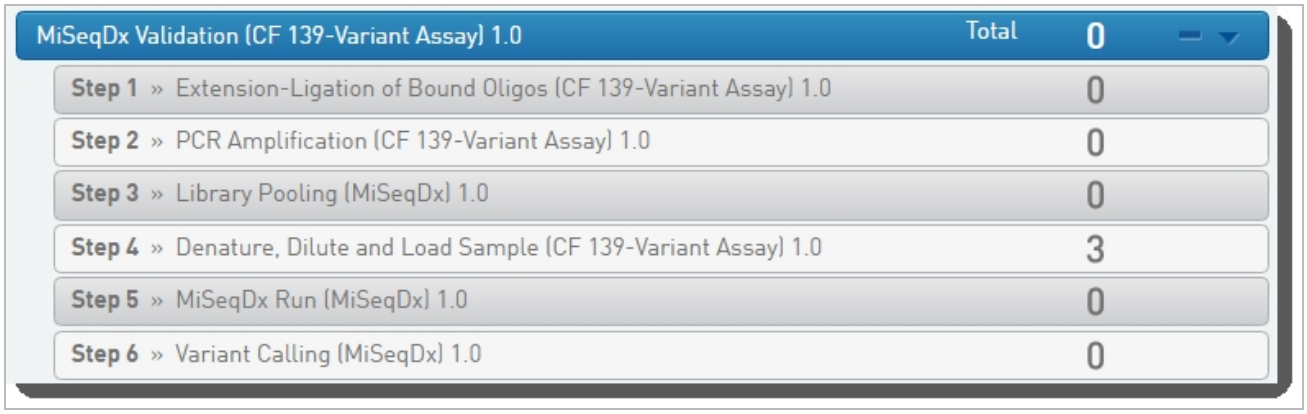


Figure 7:MiSeqDx Validation (CF 139-Variant Assay) 1.0 protocol and steps

Note that there are three Validation protocols (only the MiSeqDx Validation (CF 139-Variant Assay) 1.0 protocol is shown above in Figure 7). Each includes the following steps from the related Library Prep and Illumina SBS MiSeqDx protocols.

1. Extension-Ligation of Bound Oligos – Library Prep step

2. PCR Amplification – Library Prep step
3. Library Pooling (MiSeqDx) – Illumina SBS MiSeqDx step
4. Denature, Dilute and Load Sample – Illumina SBS MiSeqDx step
5. MiSeqDx Run (MiSeqDx) – Illumina SBS MiSeqDx step
6. Variant Calling (MiSeqDx) – Illumina SBS MiSeqDx step

For instructions on using the MiSeqDx Validation (CF 139-Variant Assay) 1.0 protocol to validate automated sample sheet generation, see the Illumina MiSeqDx Integration v1.5 Validation and Troubleshooting Guide.

Automations

Table 1 lists the automations included in this integration, and the protocol steps on which they are configured. Additional details are provided in the sections following the table.

Table 1: Illumina MiSeqDx Integration out-of-the-box protocols and automations

Protocol	Automations
• CF 139-Variant Assay Library Prep 1.0 • Universal Kit Library Prep 1.0	On PCR Amplification (CF 139-Variant Assay / Universal Kit) 1.0 step: • Auto Place Indexes • Validate Index Placement
• Illumina SBS MiSeqDx (CF 139-Variant Assay) 1.0 • Illumina SBS MiSeqDx (CF Clinical Sequencing Assay) 1.0 • Illumina SBS MiSeqDx (Universal Kit) 1.0	On Library Normalization (MiSeqDx) 1.0 step: • Create Normalization CSV
• Illumina SBS MiSeqDx (CF 139-Variant Assay) 1.0 • Illumina SBS MiSeqDx (CF Clinical Sequencing Assay) 1.0 • Illumina SBS MiSeqDx (Universal Kit) 1.0 • MiSeqDx Validation (CF 139-Variant Assay) 1.0 • MiSeqDx Validation (CF Clinical Sequencing Assay) 1.0 • MiSeqDx Validation (Universal Kit) 1.0	On Denature, Dilute and Load Sample (CF 139-Variant Assay / CF Clinical Sequencing Assay / Universal Kit) 1.0 step: • Generate MiSeqDx Sample Sheet • Validate Container Name

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Protocol	Automations
• Illumina SBS MiSeqDx (CF 139-Variant Assay) 1.0 • Illumina SBS MiSeqDx (CF Clinical Sequencing Assay) 1.0 • Illumina SBS MiSeqDx (Universal Kit) 1.0 • MiSeqDx Validation (CF 139-Variant Assay) 1.0 • MiSeqDx Validation (CF Clinical Sequencing Assay) 1.0 • MiSeqDx Validation (Universal Kit) 1.0	On MiSeqDx Run (MiSeqDx) 1.0 step: • AUTOMATED - Run Report Generation

The following sections describe how these automations work, and how the lab scientist interacts with them in the lab.

Note:
As of BaseSpace Clarity LIMS v5.0, the term user-defined field (UDF) has been replaced with custom field in the user interface (the API resource is still called udf). There are two types of custom fields:

- Master step fields - configured on master steps. These fields only apply to the master step on which they are configured, and the steps derived from those master steps.
- Global fields - configured on entities such as submitted sample, derived sample, measurement, etc. These fields apply to the whole LIMS system.

Library Prep protocols

This section discusses the index placement and validation automations (configured on PCR Amplification 1.0 Library Prep step), and describes the user interaction involved in running samples through Library Prep steps.

The example workflow below uses the CF 139-Variant Assay Library Prep 1.0 protocol. However, important considerations related to the Universal Kit Library Prep 1.0 and CF Clinical Sequencing Assay Library Prep 1.0 protocols are noted.

A list of reagents installed with this configuration is provided in the Components installed with this integration section of this article.

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Best Practices Note

Good laboratory practices mandate that a positive control DNA sample and a negative (no-template) control sample are included in every run. See sub-step 2 of Step 1. Hybridization of Oligo Pool (CF 139-Variant Assay) 1.0 section below. The positive control DNA sample should be a well-characterized sample with a known CFTR mutation.

Auto-placement of reagent indexes

By default, in the CF 139-Variant Assay Library Prep 1.0 and the Universal Kit Library Prep 1.0 protocols, the PCR Amplification 1.0 step includes two automations (both of which invoke the place_ indexes script with different options):

- Auto Place Indexes is automatically invoked on entry to the Add Reagents screen.
- Validate Index Placement is automatically invoked on exit from the Add Reagents screen.

Figures 8 and 9 show these automations configured on the PCR Amplification 1.0 step, in LIMS v4.x and v5.x.

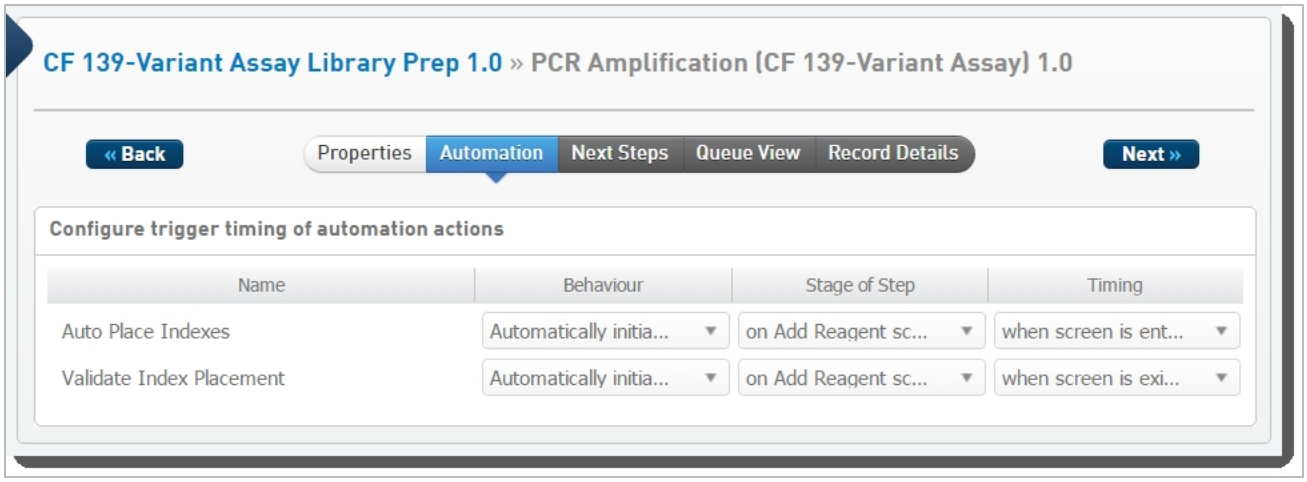


Figure 8: Configuration screen showing automations configured on PCR Amplification (CF 139-Variant Assay) 1.0 step in LIMS v4.x

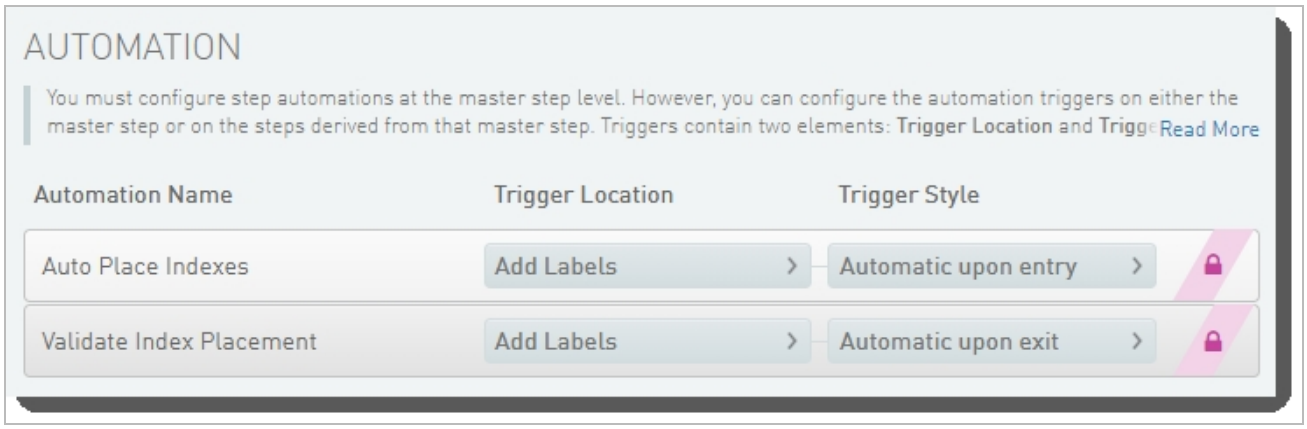


Figure 9: Configuration screen showing automations configured on PCR Amplification (CF 139-Variant Assay) 1.0 step in LIMS v5.x

How it works

On entry to the Add Reagents screen, the Auto Place Indexes automation is invoked. This in turn invokes the place_indexes script, which places reagent indexes into the container based on the pattern defined in a specified index placement pattern file (Table 2 shows the pattern file names and their location).

1. Reagent indexes are assigned to each sample in the destination container according to the specified index placement pattern file. Note the following: The automatic index placement pattern for Universal Kit may be modified.
    - The automatic index placement pattern for CF 139-Variant Assay should not be modified.
    - If an 8-sample reagent category / label group is selected (see step 1 of User interaction section below), index placement must be performed manually.

    Note:
    Index placement must be performed manually for the CF Clinical Sequencing Assay Library Prep 1.0 protocol, which only uses 8-sample reagent categories / label groups.

    - Upon completion of the reagent index placement, a success message displays. User clicks OK to close the message. Indexes with the pattern specified in the placement file are then assigned to the samples.
2. On exit from the Add Reagents screen, the Validate Index Placement automation invokes the place_indexes script once again, this time to validate the index placement:
    - If the index placement is user-modifiable (Universal Kit only), any changes made by the user are preserved.
    - If the index placement cannot be modified (CF 139-Variant Assay), any changes made by the user are disregarded and the automatic index placement is restored.

Table 2 shows the commands configured on the Auto Place Indexes and Validate Index Placement automations.

Table 2: PCR Amplification 1.0 step automation details

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PCR Amplification (CF 139-Variant Assay) 1.0 step:

Automation	Trigger	Command
Auto Place Indexes	On entry to Add Reagent sscreen	bash -l -c "/opt/gls/clarity/bin/java -cp /opt/gls/clarity/extensions/ngs-common/v5/EPP/PlacementHelper.jar place_indexes -i {stepURI:v2} -u {username} -p {password} -f /opt/gls/clarity/extensions/conf/miseqdx/placementpatterns/placement_pattern_index_cf139_01.tsv -ic 'CF 139-Variant Assay 8-Sample Indexes' -locked true"
Validate Index Placement	On exit from Add Reagent sscreen	bash -l -c "/opt/gls/clarity/bin/java -cp /opt/gls/clarity/extensions/ngs-common/v5/EPP/PlacementHelper.jar place_indexes -i {stepURI:v2} -u {username} -p {password} -f /opt/gls/clarity/extensions/conf/miseqdx/placementpatterns/placement_pattern_index_cf139_01.tsv -ic 'CF 139-Variant Assay 8-Sample Indexes' -locked true -fc true"

PCR Amplification (Universal Kit) 1.0 step:

Automation	Trigger	Command
Auto Place Indexes	On entry to Add Reagent sscreen	bash -l -c "/opt/gls/clarity/bin/java -cp /opt/gls/clarity/extensions/ngs-common/v5/EPP/PlacementHelper.jar place_indexes -i {stepURI:v2} -u {username} -p {password} -f /opt/gls/clarity/extensions/conf/miseqdx/placementpatterns/placement_pattern_index_universal_01.tsv -ic 'Universal Kit 8-Sample Indexes'"
Validate Index Placement	On exit from Add Reagent sscreen	bash -l -c "/opt/gls/clarity/bin/java -cp /opt/gls/clarity/extensions/ngs-common/v5/EPP/PlacementHelper.jar place_indexes -i {stepURI:v2} -u {username} -p {password} -f /opt/gls/clarity/extensions/conf/miseqdx/placementpatterns/placement_pattern_index_universal_01.tsv -ic 'Universal Kit 8-Sample Indexes' -fc true"

User interaction

1. Run the PCR Amplification (CF 139-Variant Assay) 1.0 step, add samples to the Ice Bucket and select Reagent Label Options : For manual placement of indexes, select CF 139-Variant Assay 8-Sample Indexes.- or -
    - For automatic placement of indexes, select CF 139-Variant Assay Indexes.
    - If you are working with 8 or less samples:
    - If you are working with more than 8 samples, select CF 139-Variant Assay Indexes for automatic placement of indexes.
2. Click Begin Work.

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3. On the Record Details screen, click Add Reagents.
4. A message box displays, notifying you that a custom script is in progress. Upon script completion, a success message displays. Click OK to proceed.
5. Indexes with the pattern specified in the placement file are assigned to samples and displayed.
    - If running the Universal Kit protocol, you may modify the index placement.
    - If running the CF 139-Variant Assay protocol, index placement is not modifiable. If you attempt to modify the placement pattern, your changes will be reverted on exit from the Add Reagents screen and the automatic placement will be restored.
6. Click Next Steps.
7. The LIMS does the following:
    - Validates the index placement.
    - In the CF 139-Variant Assay protocol, reverts any changes made to index placement.
    - Displays a success message. Click OK to proceed.

Step 1 - Hybridization of Oligo Pool (CF 139-Variant Assay) 1.0

User interaction

1. Add samples to Ice Bucket.
2. Add Negative Control and Positive Control samples to Ice Bucket (seeBest Practices Noteabove).

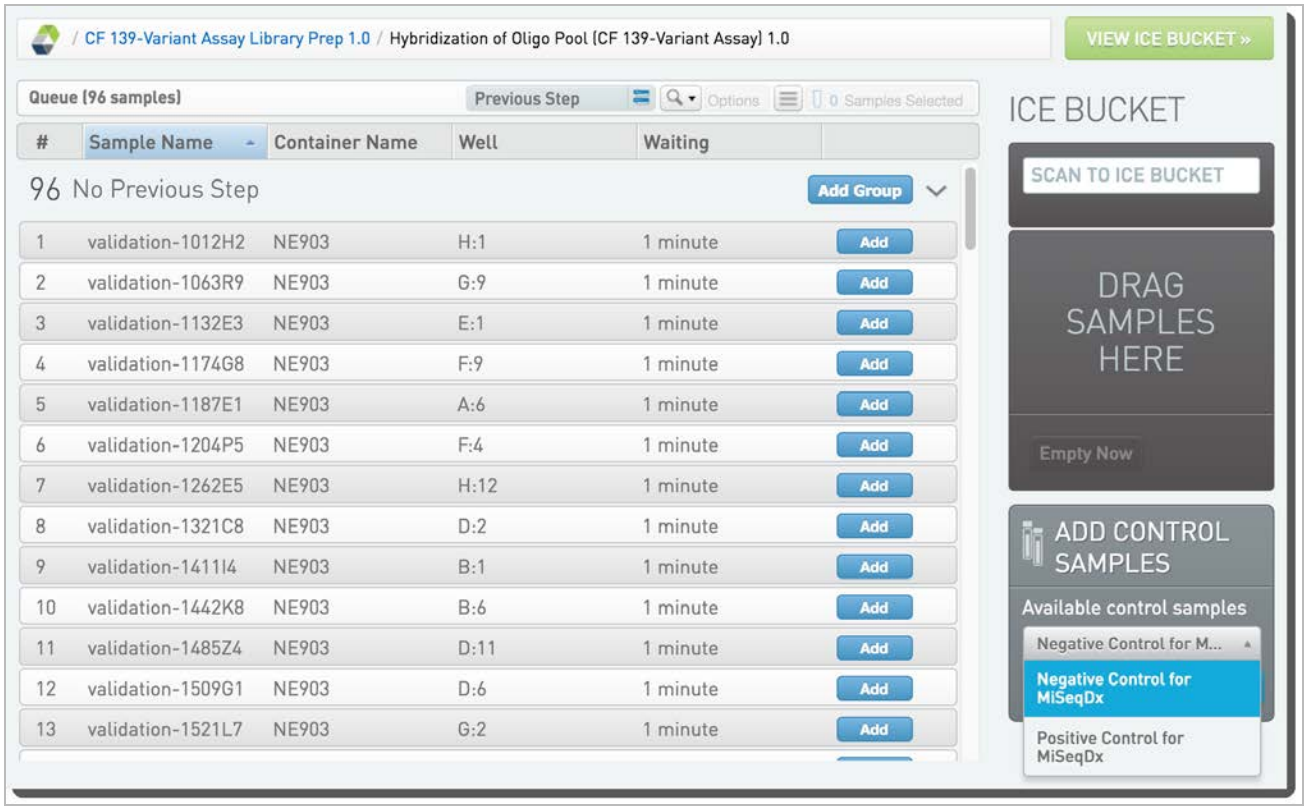


Figure 10: Adding control samples to the Ice Bucket

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3. Click Begin Work.
4. On the Placement screen, select all samples and place in container. Click Record Details.
5. On the Record Details screen, under Reagent Lot Tracking :gDNA Concentration (ng/uL): 50 ng/ul
    - gDNA Volume (uL): 5 uL
    - Select the reagent lots for CF 139-Variant Assay-Oligo Pool and Hybridization Buffer.

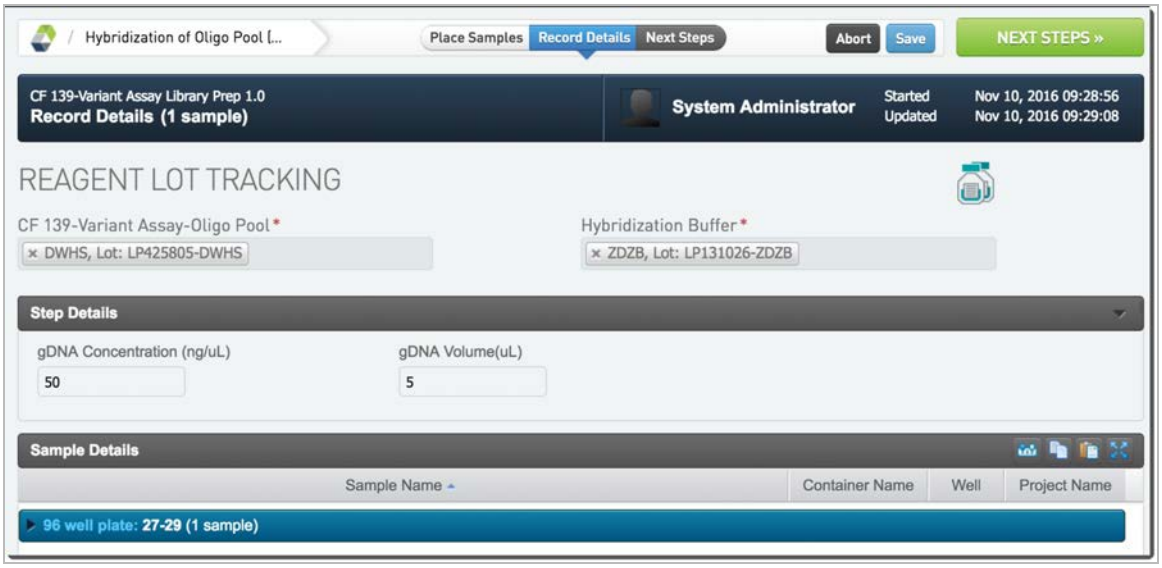


Figure 11: Adding reagents in Hybridization of Oligo Pool (CF 139-Variant Assay) 1.0 step

    - Note that the following default values are set automatically:
    - Click Next Steps.
6. Select Removal of Unbound Oligos (CF 139-Variant Assay) 1.0 as the next step. Click Apply.
7. Click Finish Step.

Step 2 - Removal of Unbound Oligos (CF 139-Variant Assay) 1.0

User interaction

1. Add samples to Ice Bucket. Click Begin Work.
2. On the Placement screen, select all samples and place in container.
3. Click Record Details.

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4. On the Record Details screen, under Reagent Lot Tracking, select the reagent lots for Stringent Wash Buffer and Universal Wash Buffer.

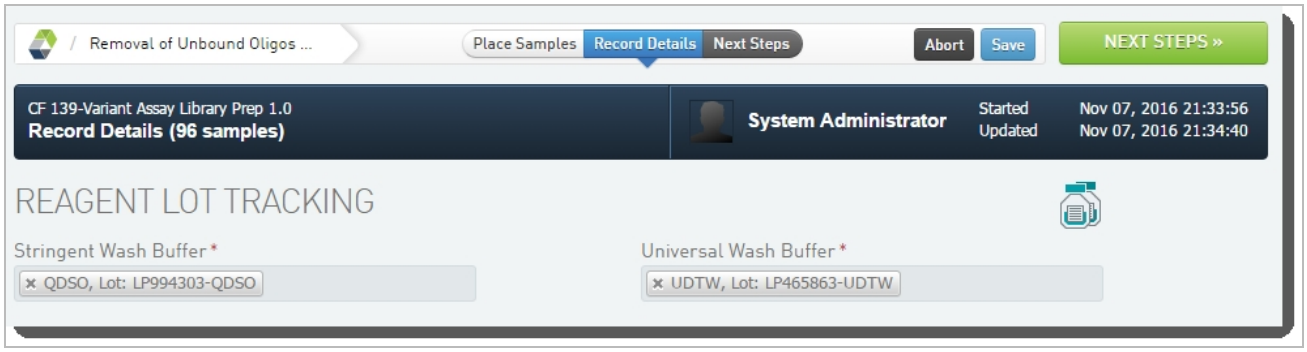


Figure 12: Adding reagents in Removal of Unbound Oligos (CF 139-Variant Assay) 1.0 step

5. Click Next Steps.
6. Select Extension-Ligation of Bound Oligos (CF 139-Variant Assay) 1.0 as the next step. Click Apply.
7. Click Finish Step.

Step 3 - Extension-Ligation of Bound Oligos (CF 139-Variant Assay) 1.0

User interaction

1. Add samples to Ice Bucket. Click Begin Work.
2. On the Placement screen, select all samples and place in container.
3. Click Record Details.
4. On the Record Details screen, under Reagent Lot Tracking, select the reagent lots for Extension-Ligation Mix.

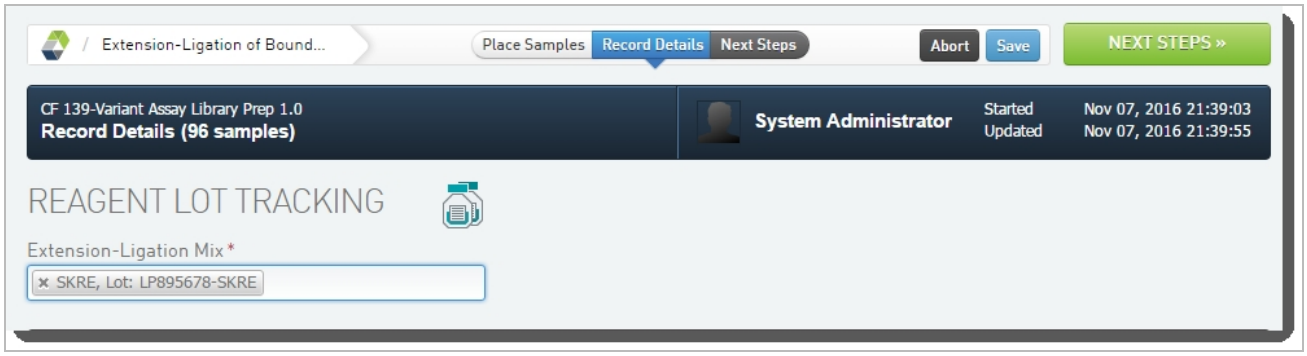


Figure 13: Adding reagents in Extension-Ligation of Bound Oligos (CF 139-Variant Assay) 1.0 step

5. Click Next Steps.
6. Select PCR Amplification (CF 139-Variant Assay) 1.0 as the next step. Click Apply.
7. Click Finish Step.

Step 4 - PCR Amplification (CF 139-Variant Assay) 1.0

User interaction

1. Add samples to Ice Bucket.

2. Select Reagent Label Options:
    - If you are working with 8 or less samples, select CF 139-Variant Assay 8-Sample Indexes.
    - If you are working with more than 8 samples, select CF 139-Variant Assay Indexes.

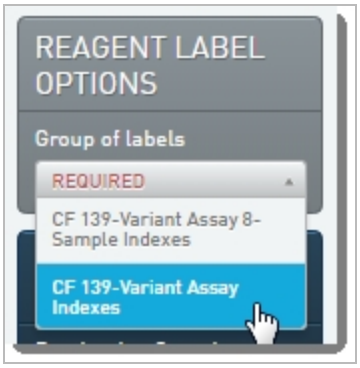


Figure 14: Selecting Reagent Label Options

    - Click Begin Work.
3. On the Placement screen, select all samples and place in container. Click Add Reagents.
4. On entry to the Add Reagents screen, the Auto Place Indexes automation is invoked and the reagents are placed on the samples.
5. Once placement is complete, a message displays, indicating that the index pattern has been applied successfully. Click OK.

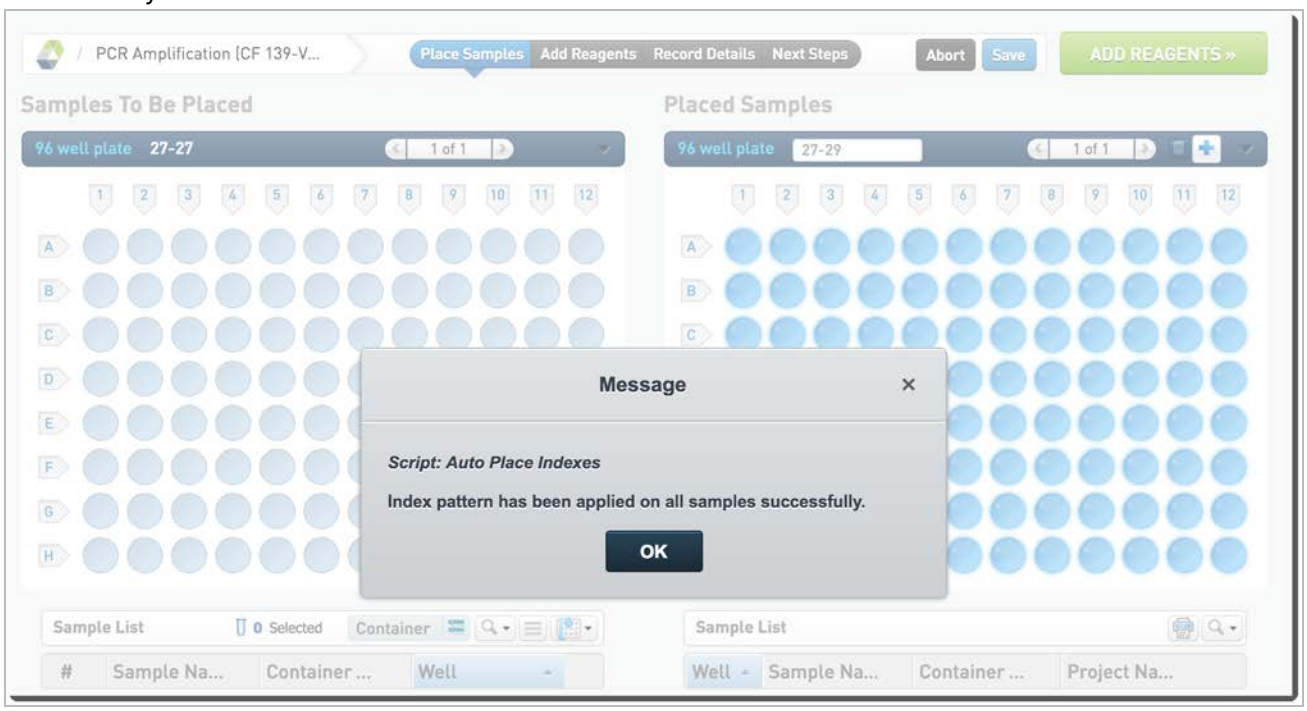


Figure 15: Index pattern placement success message in PCR Amplification (CF 139-Variant Assay) 1.0 step

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6. On Add Reagents screen, reagents are already placed. Click Record Details.

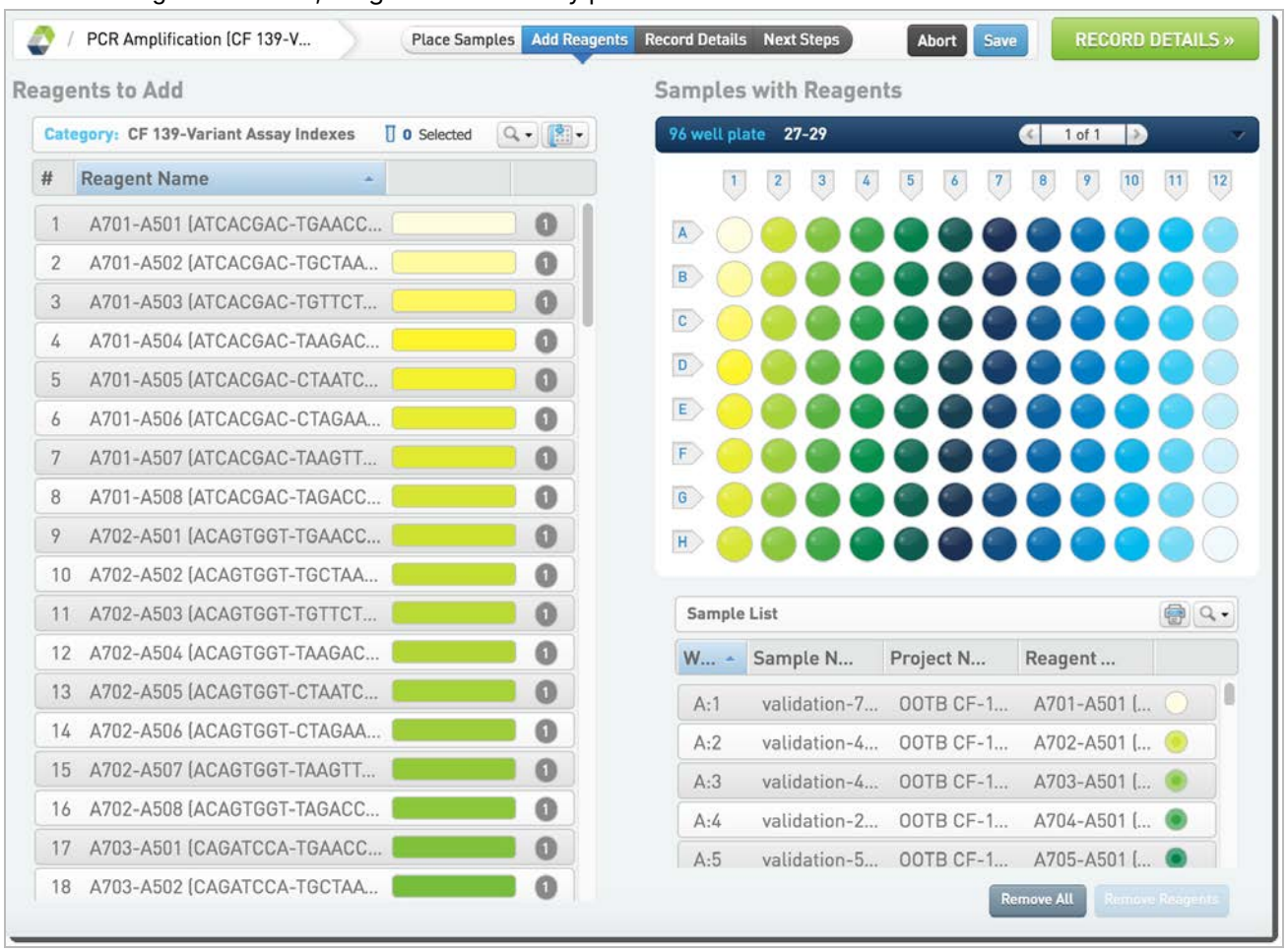


Figure 16: Reagents are automatically placed in PCR Amplification (CF 139-Variant Assay) 1.0 step

7. Click Record Details.
8. On exit from the Add Reagents screen, the index placement is validated. Once validation is complete, a message displays, indicating that the index pattern has been validated on all samples successfully. Click OK.

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9. On the Record Details screen, under Reagent Lot Tracking, enter the lots for Index Primers, NaOH, PCR Master Mix, and PCR Polymerase.

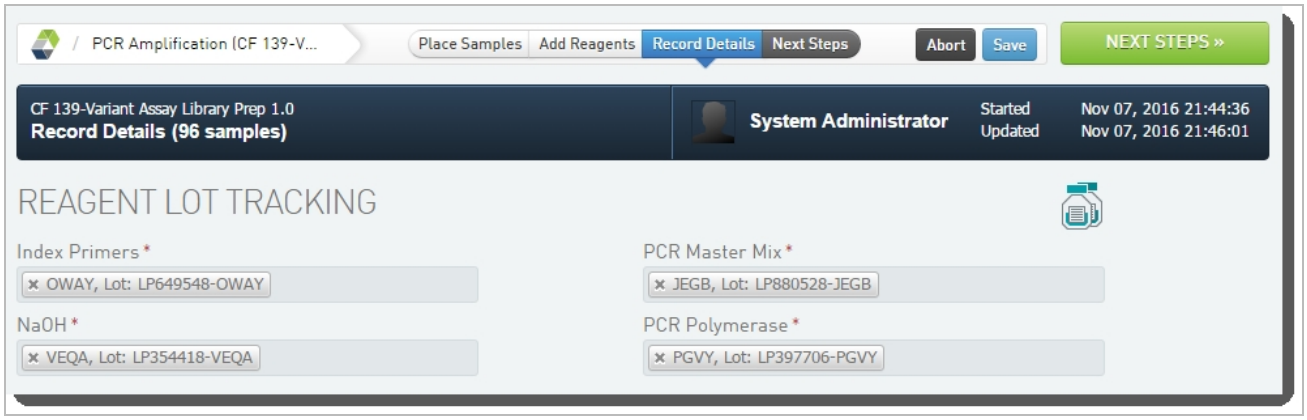


Figure 17: Adding reagents in PCR Amplification (CF 139-Variant Assay) 1.0 step

10. Click Next Steps.
11. Select PCR Clean-Up (CF 139-Variant Assay) 1.0 as the next step. Click Apply.
12. Click Finish Step.

Step 5 - PCR Clean-Up (CF 139-Variant Assay) 1.0

User interaction

1. Add samples to Ice Bucket. Click Begin Work.
2. On the Placement screen, select all samples and place in container. Click Record Details.
3. On the Record Details screen, under Reagent Lot Tracking, select the reagent lots for Elution Buffer, EtOH and PCR Clean-up Beads.

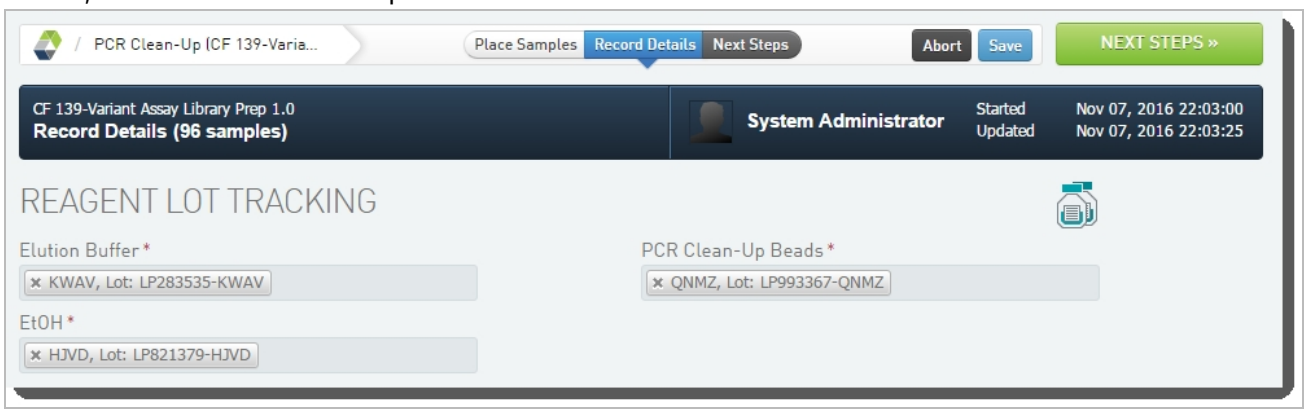


Figure 18: Adding Reagents in PCR Clean-Up (CF 139-Variant Assay) 1.0 step

4. Click Next Steps.
5. Select Mark protocol as complete as the next step. Click Apply.
6. Click Finish Step.

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Illumina SBS MiSeqDx protocols

The sections below describe the following key features of the Illumina SBS MiSeqDx protocols, and the user interaction involved at those points in the workflow:

- Library normalization CSV file generation
- Reagent cartridge name validation
- MiSeqDx sample sheet generation
- Primary analysis (sequencing) results parsing, which includes generation of the run report
- Secondary analysis results parsing

Library normalization file generation

In each of the Illumina SBS MiSeqDx protocols, the Library Normalization (MiSeqDx) 1.0 step includes automated calculation of normalization buffer volumes. The results are provided in auto-generated comma-separated file that is attached to the step.

How it works

Buffer volumes are calculated by the normalizationBufferVolumes script. The script is invoked by the Create Normalization CSV automation when the user clicks a button on the Record Details screen.

For details on the normalizationBufferVolumes script, see Normalization Buffer Volumes.

User interaction

On the Record Details screen of the Library Normalization (MiSeqDx) 1.0 step:

1. Enter values for each of the following:
    - Library volume (uL) transferred to destination plate
    - Normalized conc (nM)
    - Maximum destination container volume (uL)
    - Any other required custom fields / UDFs

2. Click Create Normalization CSVto invoke the automation.

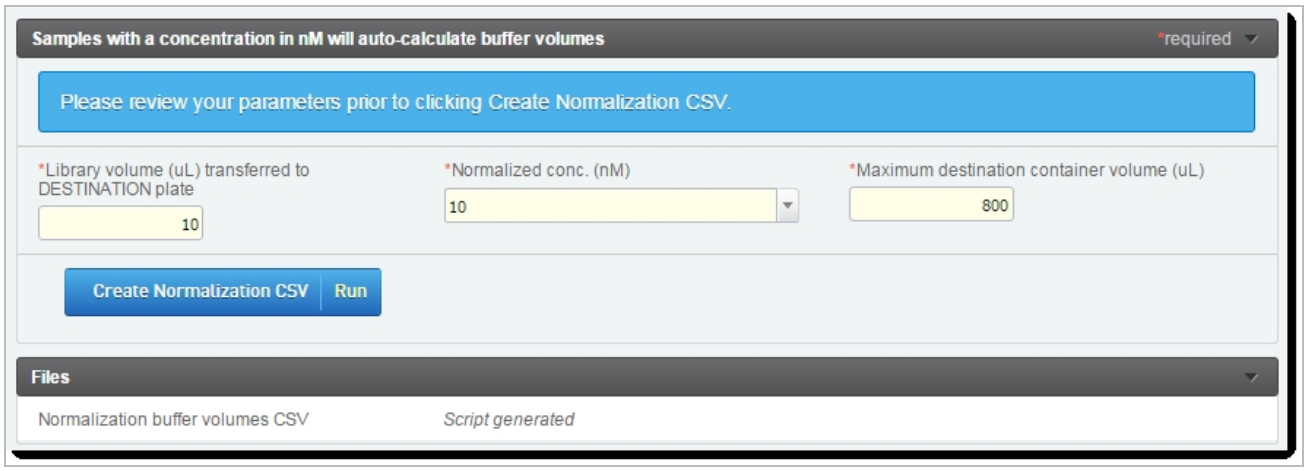


Figure 19: Record Details screen of Library Normalization (MiSeqDx) 1.0 step Once the normalizationBufferVolumes script has completed, the Normalization buffer volumes CSV file is generated and attached to the step – in the Files area of the Record Details screen.

Reagent cartridge name validation

In each of the Illumina SBS MiSeqDx and Validation protocols, the Denature, Dilute and Load Sample 1.0 step includes automated validation of the MiSeqDx reagent cartridge name.

How it works

Automatically initiated on exit from the Placement screen of the step, the Validate Container Name automation validates the reagent cartridge name to ensure:

- The reagent cartridge Container Name has been renamed and is not the same as its LIMS ID.
- The Container Name contains only letters and numbers, and no special characters, and is in the format AB########-XYZ (e.g., RG1234567-ABC).

Figures 20 and 21 show the Validate Container Name automation configured on the Denature, Dilute and Load Sample (CF 139-Variant Assay) 1.0 step, in LIMS v4.x and v5.x.

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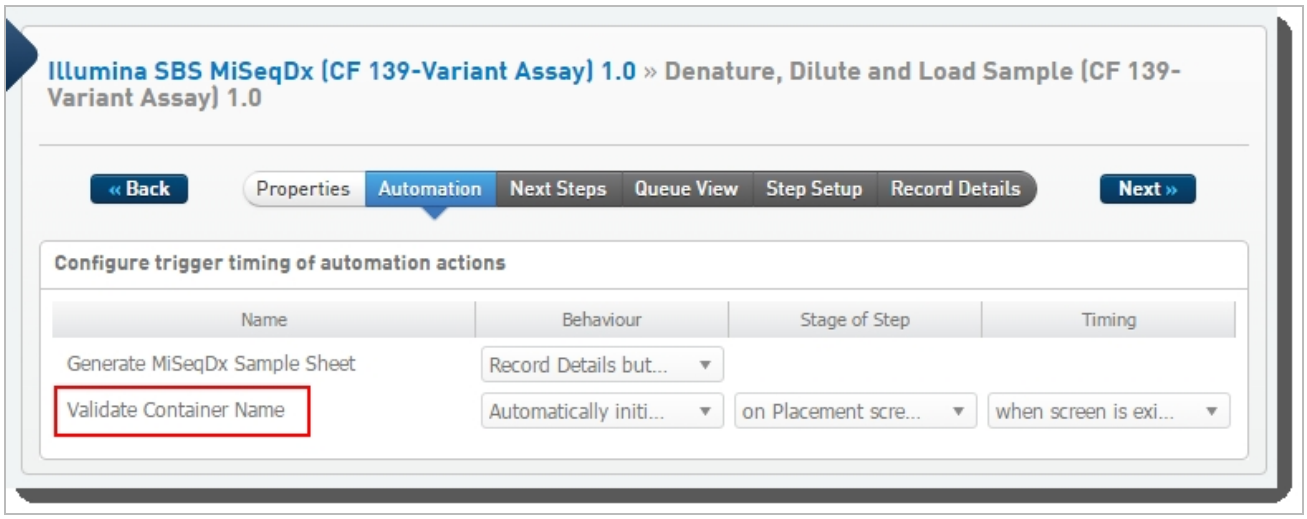


Figure 20: Configuration screen showing Validate Container Name automation configured on Denature, Dilute and Load Sample 1.0 step in LIMS v4.x

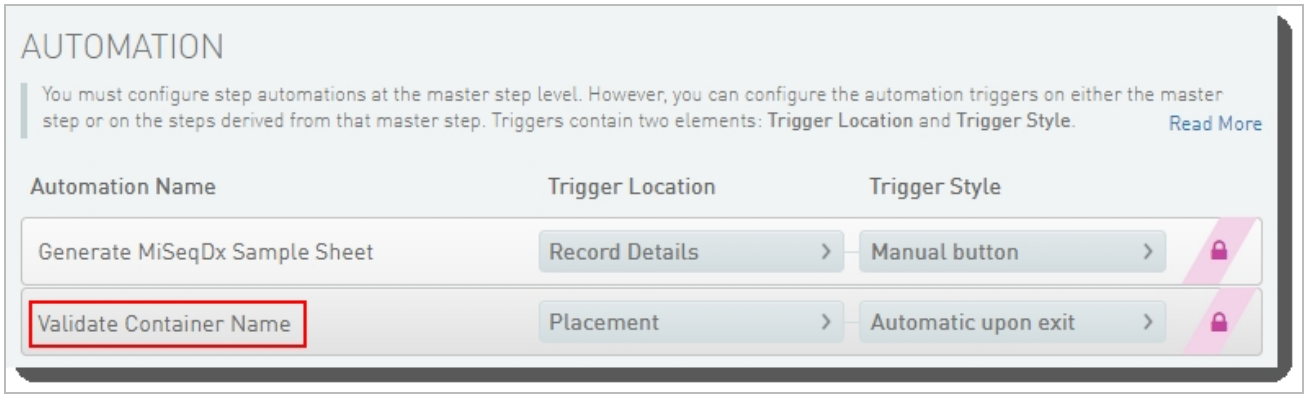


Figure 21: Configuration screen showing Validate Container Name automation configured on Denature, Dilute and Load Sample 1.0 step in LIMS v5.x

User interaction

1. On the Placement screen of the Denature, Dilute and Load Sample 1.0 step:
    - Scan the RFID of the MiSeqDx reagent cartridge into the Container Name field, using an RFID scanner.
    - Place the pool of samples into the reagent cartridge.
    - Click Record Details.
2. On exit from the Placement screen, the automation is automatically triggered and validates the Container Name.

Sample sheet generation

In each of the Illumina SBS MiSeqDx and Validation protocols, automated sample sheet generation is configured on the Record Details screen of the Denature, Dilute and Load Sample 1.0 step – this is when the samples are loaded onto the container that will be placed in the instrument.

The integration allows for generation of a sample sheet designed to be used with the MOS (Diagnostic mode) instrument software.

By default, one MiSeqDx instrument sample sheet is created for the reagent cartridge loaded during the step.

Table 3: Placeholders and sample sheet files generated by Denature, Dilute and Load Sample 1.0step

Item	Description
Lab Tracking Formplaceholder	This placeholder allows the user to manually attach a lab-specific tracking form to the step.
MiSeqDxSample Sheet file	This CSV file is automatically generated by the LIMS for use with the MOS or MCS software. • May be opened as a text file, or as an MS Excel spreadsheet
MiSeqDx Sample Sheet Generation Log	Automatically generated by the LIMS, this log file captures any errors encountered when generating the MiSeqDx sample sheet (MOS).

How it works

The sample sheet generation automation is configured on the Record Details screen of the Denature, Dilute and Load Sample 1.0 step.

This automation:

- Is triggered by a button on the Record Details screen.
- Invokes a script that generates the sample sheet – a comma-separated text file containing the required columns.

Note:
To maintain unique values in the Sample_Name column of the sample sheet, the sample sheet generation automation script has the useSampleLimsID parameter set to true:
useSampleLimsID = true
Note also that the appendLimsID parameter must not be set to true.

For Research Use Only. Not for use in diagnostic procedures.

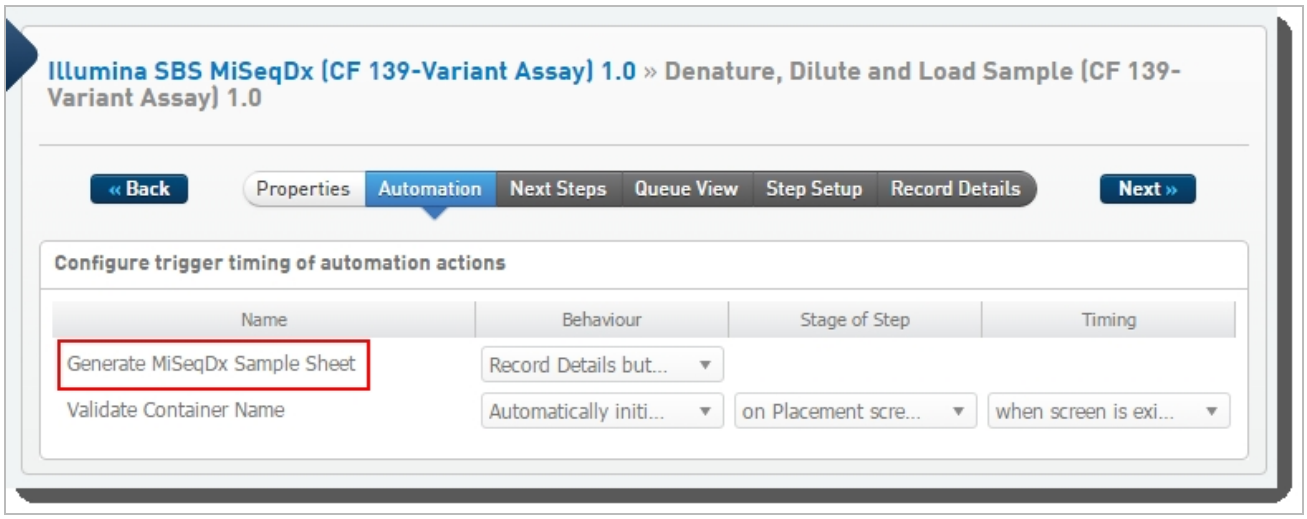


Figure 22: Configuration screen showing Generate MiSeqDx Sample Sheet automation configured on Denature, Dilute and Load Sample 1.0 step in LIMS v4.x

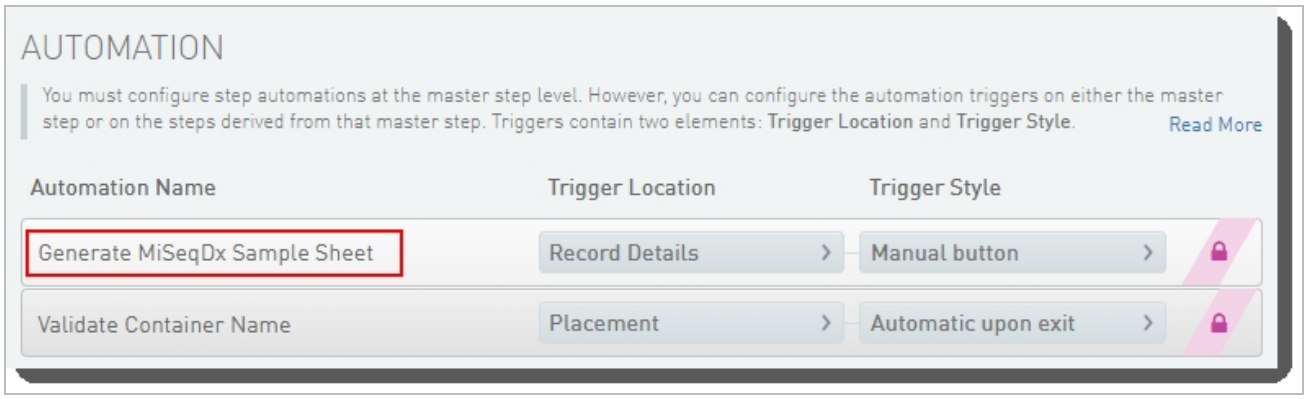


Figure 23: Configuration screen showing Generate MiSeqDx Sample Sheet automation configured on Denature, Dilute and Load Sample 1.0 step in LIMS v4.x

Supporting documentation

For more information on sample sheet generation, including details on file format and contents, master step fields / step UDFs and submitted sample global fields / sample UDFs, script parameters, and configuration options, see the following articles:

- For details on the Diagnostic mode sample sheet designed for use with MOS software, see MiSeqDx 1.x Sample Sheet Generation.
- For details on the Research mode sample sheet designed for use with MCS software, see MiSeq Sample Sheet Generation.

User interaction

1. On the Record Details screen of the Denature, Dilute and Load Sample 1.0 step:
    - All read-only fields are auto-populated.

- GenomeFolder is a required field. Enter the folder path containing the Reference Genomes that will be used for secondary analysis here.
- In the Reagent Lot Tracking section, select the reagent lots used in the step. Enter the lots for MiSeqDx Flow Cell - CF 139-Variant Assay and MiSeqDx SBS Solution (PR2) - CF 139-Variant Assay.
- In the Step Details section, review the step parameters:
- Click Generate MiSeqDx Sample Sheet to generate the sample sheet to be uploaded to the MiSeqDx instrument.
- Once the automation has completed, the MiSeqDx sample sheet and log file are generated and attached to the step.
- Download the sample sheet and upload it to the MiSeqDx instrument to start a run.

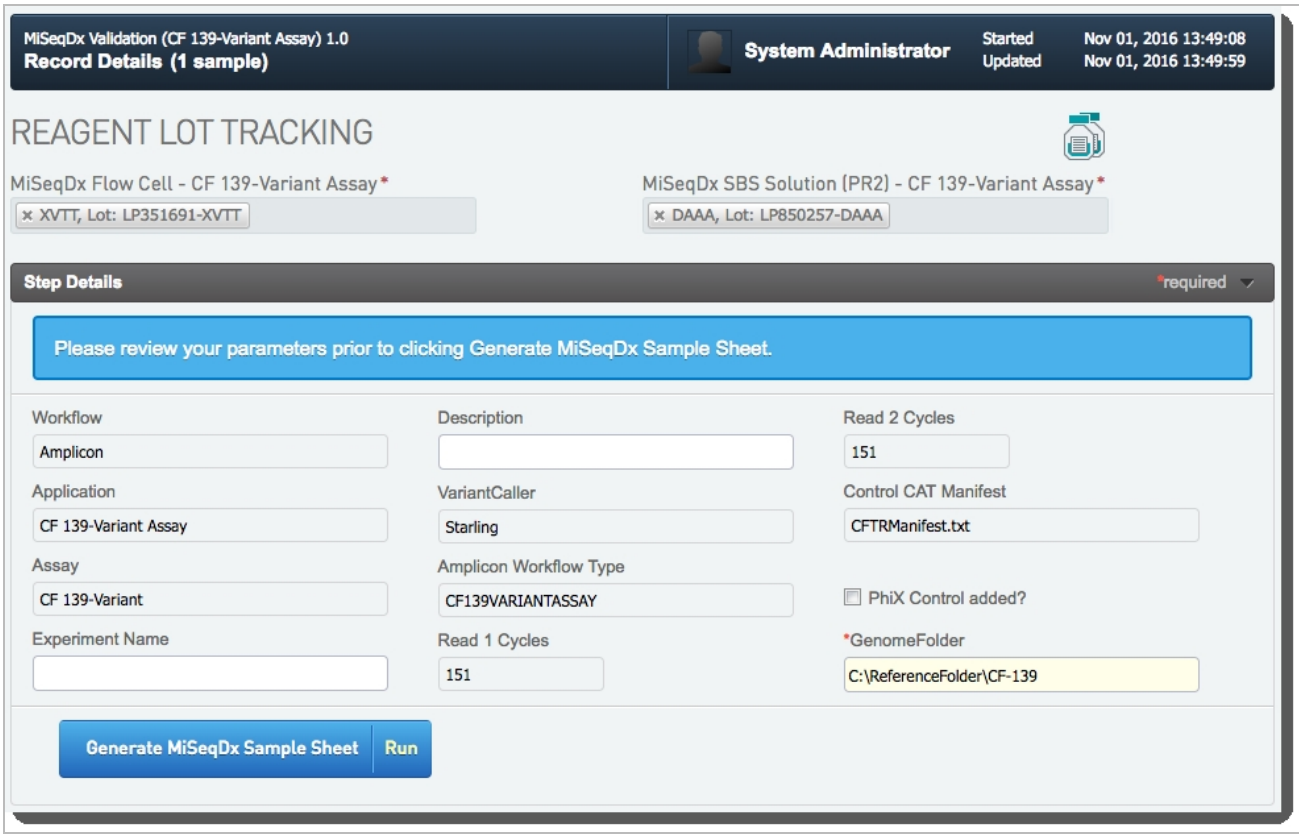


Figure 24: Record Details screen of Denature, Dilute and Load Sample 1.0 step

- (Optional) In theFiles section, upload the Lab Tracking Formto the appropriate placeholder.



Figure 25: Files section of Record Details screen showing attached MiSeqDx Sample Sheet and Sample Sheet Generation Log files and Lab Tracking Form placeholder

- Click Next Steps.

2. On the Assign Next Steps screen, assign the samples to the MiSeqDx Run (MiSeqDx) 1.0 and click Finish Step.

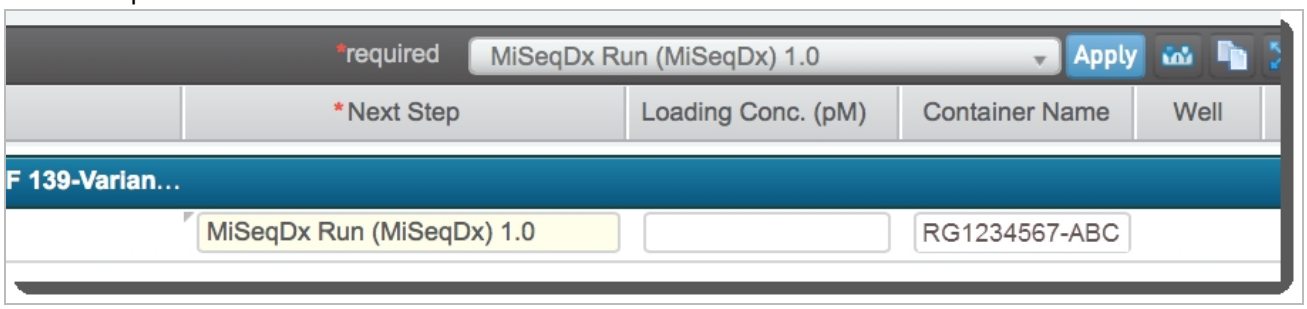


Figure 26: Assigning samples to the MiSeqDx Run (MiSeqDx) 1.0 step

Primary analysis (sequencing) results parsing

The MiSeqDx Run (MiSeqDX) step records information for each lane of the flow cell and generates a report summarizing the results. In addition, run parameters, run info, and a link to the run folder are automatically captured.

Generated and captured files

Table 4 describes the run information files, reports, placeholders, and links that the LIMS automatically generates or captures during a sequencing run.

Table 4: Run information generated or captured by MiSeqDx Run (MiSeqDX)1.0 step

Item	Description
Run Info Run Parameters	These XML files are automatically captured by the LIMS from the instrument's run folder. They include the key run parameters, many of which are parsed out into key master step fields / step UDFs.
Link to Run Folder	Automatically generated by the LIMS, this is a link to the network run folder where the data that was captured from the instrument during the run is stored.

Item	Description
Illumina Run Report	Automatically generated by the LIMS, this report provides key information about the run and the samples on the flow cell. Information includes the flow cell ID, run directory location, instrument name, as well as primary analysis metrics for the instrument – summarized per flow cell lane for the entire run, and individual reads in the case of paired-end runs. These metrics are compared against the instrument's per lane averages, calculated using metrics from the last 5 sequencing runs. Any values outside of 1 standard deviation are highlighted. Note: The run report calculations assume that the flow cell has eight lanes. If you are using a two-lane rapid run flow cell, the calculated values will be incorrect.
Lab Tracking Form placeholder	This placeholder in the LIMS allows the user to manually attach a lab-specific tracking form to the step.

Metadata

- Date Run* – run start date
- Instrument Run ID – sequencer instrument name
- Control Lane – PhiX control lane (blank or 1-8)
- Experiment Name – entered in software
- Finish Date* – run completion date
- Flow Cell ID
- Flow Cell Position – A or B
- Flow Cell Version
- Output Folder – run folder root
- Index 1 Read Cycles – intended Index cycles
- Index 2 Read Cycles – intended Index cycles
- Run ID – the unique run ID
- Run Type – Diagnostic or Research mode
- Status – current status of the sequencing run on the instrument
- SBS Kit Lot #
- SBS Kit Type

Note:Date Run is populated with the date that the Begin Run event file is first picked up and associated with a step in the LIMS, not the date of the Begin Run event itself (the date the run was performed on the instrument).

Finish Date is populated as follows:

- If the End Run event contains a date in the format YYYY-MM-DD, Finish Date will be set to the date in the event file
- If the End Run event does not contain a date or the date is in the wrong format, Finish Date will be set to the date when the event file is processed

Primary analysis metrics

Table 5 lists the Real Time Analysis (RTA) primary analysis metrics the LIMS automatically captures and records, per read, for samples in each flow cell lane. These metrics are captured upon run completion.

Table 5: RTA primary analysis metrics captured by MiSeqDx Run (MiSeqDX)step

RTA Primary Analysis Metrics	Corresponding LIMS custom fields / UDFs
Raw Yield (Gb)	Yield PF (Gb) R1 Yield PF (Gb) R2
% Bases >Q30	% Bases >=Q30 R1 % Bases >=Q30 R2
Cluster Density (K/mm^2)	Cluster Density (K/mm^2) R1 Cluster Density (K/mm^2) R2
Clusters Raw	Clusters Raw R1 Clusters Raw R2
Clusters PF	Clusters PF R1 Clusters PF R2
%PF	%PF R1 %PF R2
Intensity Cycle 1	Intensity Cycle 1 R1 Intensity Cycle 1 R2
% Intensity Cycle 20	% Intensity Cycle 20 R1 % Intensity Cycle 20 R2
% Phasing	% Phasing R1 % Phasing R2
% Prephasing	% Prephasing R1 % Prephasing R2
% Aligned	% Aligned R1 % Aligned R2
% Error Rate	% Error Rate R1 % Error Rate R2

How it works

1. The sequencing service may run on the LIMS server in an on-premise environment or on a remote server in a SaaS environment. The service detects event files that the instrument software (RTA) is producing as the run progresses; this in turn lets the service know where to find the run data.
2. As the run data is written out and the events come in (Begin Run, Cycle Complete, End Run):
    - The data is matched to the step based on the reagent cartridge ID, which was entered by the user as the Container Name on the Denature, Dilute and Load Sample 1.0 step.
    - Read-only field values on the Record Detailsscreen are populated accordingly (Figure 27).
3. Run report generation:
    - Run report generation is completely asynchronous; the sequencing service does not wait for the report generation to complete before processing the next event file.
    - The report is generated by an automation script - AUTOMATED - Run Report Generation, configured on the MiSeqDx Run (MiSeqDx) 1.0 step.
    - The script is configured to be invoked by a button on the Record Details screen; however, it is automatically invoked by the sequencing service when it has finished processing the end run event.

Note: It is not intended for the user to click the AUTOMATED - Run Report Generationbutton. The run report is generated and attached to the step automatically.

- An additional shared result file placeholder is configured on the step. This is for a new log file - *Illumina Run Report Log File* - that is generated and attached to the step when the report runs (Figure 28).
- The run report generation script is part of the BaseSpaceLIMS-miseqdx-extensions package.
- The run report may be successfully generated in a hosted deployment of the LIMS, where the sequencing service is running on a remote server on the customer network.

Figure 28: Record Details screen of MiSeqDx Run (MiSeqDX) 1.0 step showing AUTOMATED - Run Report Generation button and attached script-generated files

Constraints:
Following the sequencing run, the run report will be automatically generated by the AUTOMATED - Run Report Generation automation.
Do not change this name – it is expected by the sequencing service that captures instrument run results. The base name is stored in the sequenceProcessBaseName database property (see Table 7). If this name is changed without the database property being updated, the 'flow cell ID <-> sequencing step base name' matching will fail.
Known limitation:
On the Record Details screen, if the user clicks the Next Steps button before the run report has been automatically generated and attached to the step, they will not be able to return to the step and re-generate the report (run report generation can neither be invoked by the sequencing service, nor through the user interface via the automation script.) However, the user can refer to the run metrics that are populated in the grid at the bottom of the Record Details screen.
If the user clicks the Next Steps button before data has been parsed into the step, there will be no data populated on the Record Details screen for that run.

User interaction

On the Record Details screen, do not click the AUTOMATED - Run Report Generation button. The run report is generated and attached to the step automatically. This process may take a while.

On the Record Details screen of the MiSeqDx Run (MiSeqDX) 1.0 step:

1. The read-only field values are automatically populated as the sequencer runs.
2. Once the run has completed:
    - The read-only fields shown in Figure 27 are populated.
    - The Illumina Run Report and Log File are generated and attached.
    - The Run Parameters and Run Info files are attached.
    - The Link to Run Folder is generated and attached.
    - QC flags are set on each lane.

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3. (Optional) In theFiles section, upload the Lab Tracking Form.

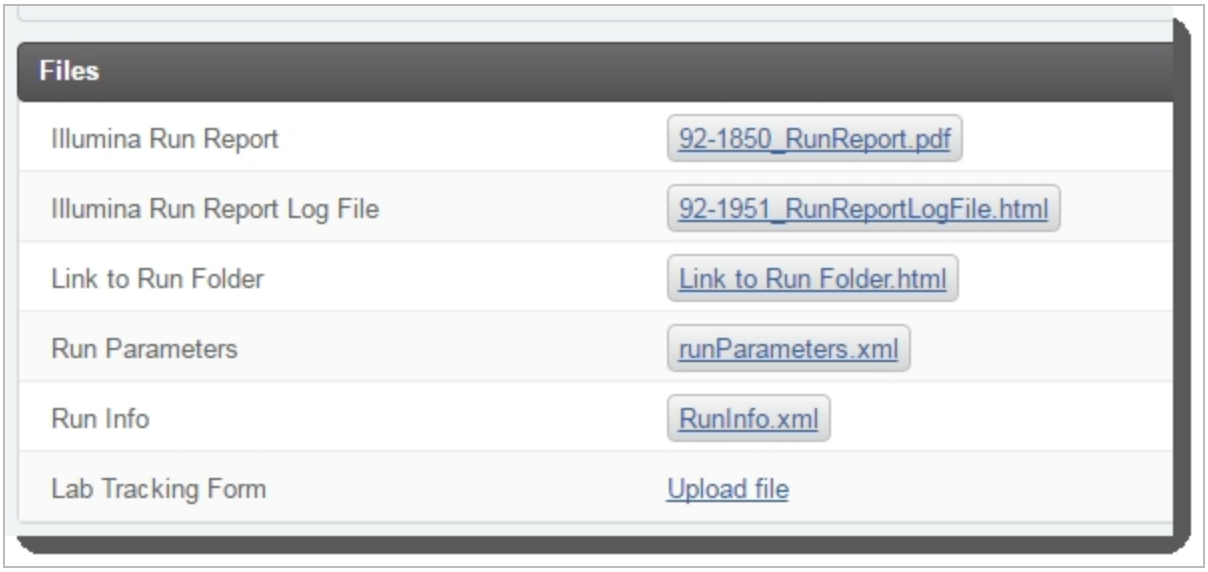


Figure 29: Record Details screen (Files section) of MiSeqDx Run (MiSeqDX) 1.0 step

4. On the Assign Next Steps screen, assign the samples to the VariantCalling (MiSeqDx) 1.0 step and click Finish Step.

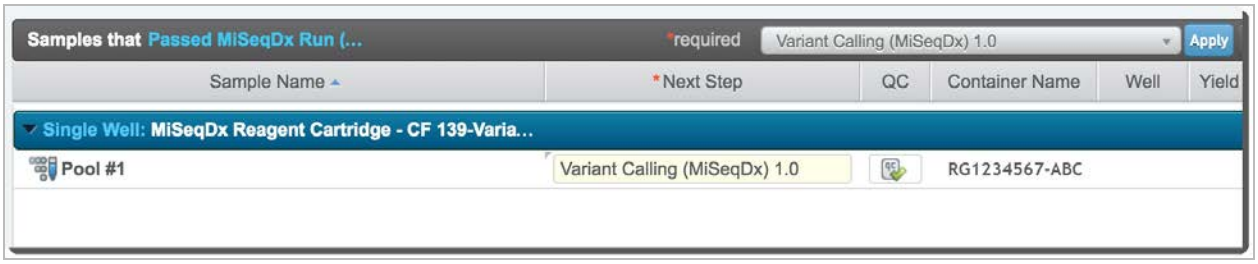


Figure 30: Assigning samples to the Variant Calling (MiSeqDx) 1.0 step

Secondary analysis output files attachment

The Variant Calling (MiSeqDX) step attaches secondary analysis output files for each of the samples.

In addition, a combined variant call file, a text file, and a variant call file for each individual sample are automatically captured following the completion of secondary analysis.

How it works

While the EndRun event is processed successfully for primary analysis, the sequence service generates a BeginSecondaryAnalysis event file in the same directory, specified by the *miseqdex.seqservice.eventFileDirectory* property.

An example of the secondary analysis event file, *event-BeginSecondaryAnalysis-14141824.txt*, has the following items:

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```
netFolder = D:\Illumina\MiSeqOutput\161017_M99999_0047_FC1234567-
ABCDErunNetworkLocation = /mnt/ussd-dev-mdx01/MiSeqOutput/161017_M99999_0047_
FC1234567-ABCDEeventType = BeginSecondaryAnalysissoftwareType = MRScreationTime =
Thu Oct 20 22:56:32 GMT 2016
```

This event file signals that secondary analysis has started. The sequence service periodically checks if the analysis is complete by looking for the appearance of the *CompletedJobInfo.xml* file in the folder specified by the *runNetworkLocation* parameter of the event file.

The frequency of checking is defined by the *miseqdx.seqservice.synchronizationPeriod* property.

- If the completion file appears, the sequence service processes the event. The event is archived in a similar way to how the EndRun event file is handled.
- If the event completion does not appear within the number days specified by the *miseqdex.seqservice.ignoreUnmatchedContainerIdsWaitDays* property, the service stops monitoring the event file and the file is archived.

Captured files

Table 6 describes the Variant Call Format (VCF) files that the LIMS captures and attaches to the step following secondary analysis.

Table 6: Variant Call Format files captured by the LIMS following secondary analysis

	Description
Combined Variant Call File	Variant call VCF files for all samples are compressed into a zip file and attached to the step for every run. Available for all 3 assays.
Combined Output Text File	For CF Clinical Sequencing Assay and CF 139-Variant Assayonly, a text file containing the summary of variant call information for all samples is attached for every run.
Variant Call File per sample	The general variant call VCF file (not CFTR VCF file) for each sample is attached. Available for all 3 assays.

How it works

Following primary analysis, secondary analysis runs on the MiSeqDx instrument. LIMS service detects the completion of secondary analysis and this in turn lets the service know where to find the result files.

User interaction

On the Record Details screen of the Variant Calling (MiSeqDX) 1.0 step:

1. The file-naming format for the VCF file is SampleName_S#.vcf, where SampleName is the value in the Sample_Name column of the sample sheet, and # is the sample number determined by the order in which samples are listed in the sample sheet.
2. Once secondary analysis has completed:
    - A Combined Variant Call File is attached to the step, this is a compressed VCF file (zip file) containing all the variant call files, including the CFTR specific VCF files (Figure 31).

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- A Combined Output Text File is attached to the step, this is a combined text file containing a summary of all the sample metrics (Figure 31).
- The Variant Call File per individual sample is attached (Figure 31).

QC flags are set on each lane (Figure 31).

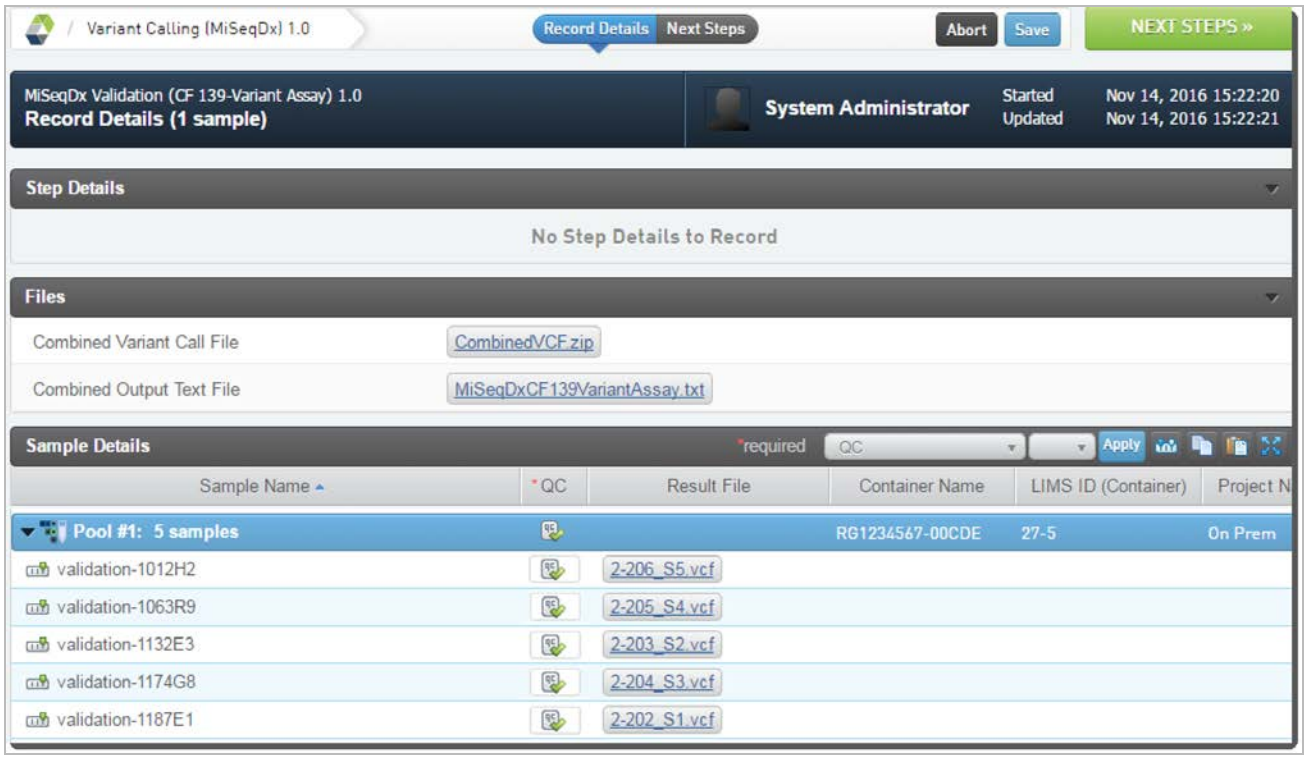


Figure 31: Variant Calling (MiSeqDX) 1.0 - QC flags set and files attached

Components installed with this integration

The Illumina MiSeqDx Integration RPM package installs the scripts and files listed in Table 7. Note that this integration requires installation of the associated NGS Extensions Package (see Release Notes).

Additional component details are provided in the four sections following the table:

- Database properties
- Reagent categories / label groups
- Reagent kits
- Controls
- Container types

Table 7: Illumina MiSeqDx Integration RPM package - scripts and files installed

Files Installed	Location and Description
configure_extensions_miseqdx_workflow.sh	/opt/gls/clarity/config/ Script that imports the integration configuration.

For Research Use Only. Not for use in diagnostic procedures.

Files Installed	Location and Description
miseqdx-extensions.jar	/opt/gls/clarity/extensions/miseqdx Jar file containing API-based LIMS extensions used throughout the protocols.
configure_extensions_ miseqdx_ sequencingservice.sh	/opt/gls/clarity/config/extensions/miseqdx Script that imports the database properties for the integration.
log4j.xml	/opt/gls/clarity/extensions/miseqdx/SequencingService/conf File containing the settings for the sequencing jar's logging.
miseqdx-sequencing-report.jar	/opt/gls/clarity/extensions/miseqdx/ Jar file containing API-based LIMS extensions used for report generation.
miseqdx-sequencing.jar	/opt/gls/clarity/extensions/miseqdx/SequencingService Sequencing service jar file that captures the run results.
miseqdx_seqservice	/opt/gls/clarity/extensions/miseqdx/SequencingService/bin Provides commands for interacting with the sequencing service. Accessible at /etc/init.d
run_miseqdx_seqservice	/opt/gls/clarity/extensions/miseqdx/SequencingService/bin Called by miseqdx_seqservice-v1, this script performs some basic system validation, then starts the sequencing service.
MiSeqDxIntegrator.log	/opt/gls/clarity/extensions/miseqdx/SequencingService Log file for Sequencing service.

Database properties installed

Table 8 lists the database properties installed with the Illumina MiSeqDx Integration Package.

Note that several additional properties, each with the '99' suffix appended to their name, are also installed. These are intended for use by the LIMS support team in automated validation tests and are not listed in the table.

Constraints

Sequencing runs are matched using the flow cell ID and the sequencing step's base name – MiSeqDx Run (MiSeqDx).

Do not change this name – it is expected by the sequencing service that captures instrument run results. The base name is stored in the sequenceProcessBaseName database property (see Table 8). If this name is changed without the database property being updated, the 'flow cell ID <-> sequencing step base name' matching system will fail.

If required, you may modify the step name by editing or adding to the text *after* the base name portion as this is not used in the matching system. For example, you could change MiSeqDx Run (MiSeqDX) 1.0 to MiSeqDx Run (MiSeqDX)v1.

Table 8: Database properties installed with the Illumina MiSeqDx Integration Package

Property	Description	Default Value
miseqdx.seqservice.ignoreUnmatchedContainerIds	A flag indicating if event files that cannot be matched to flow cells in the LIMS should be archived after a certain period of time (true), or continually reprocessed (false).	false Note: To prevent the gls_events file directory from becoming cluttered, it is recommended that the value of this property is set to 'true'.
miseqdx.seqservice.ignoreUnmatchedContainerIdsWaitDays	The number of days between when the event is created and the event file is archived.	14
miseqdx.seqservice.synchronizationPeriod	Invocation period in seconds.	60
miseqdx.seqservice.sequenceProcessBaseName	Sequencing process type base display name. Partial matching is used to look up the process type.	MiSeqDx Run (MiSeqDx)
miseqdx.seqservice.eventFileDirectory.1	A network location monitored for event files.	/mnt/gls_events
miseqdx.seqservice.netPathPrefixSearch.1	The network directory prefix contained in the event file.	\\nas\network\run_data

Property	Description	Default Value
miseqdx.seqservice.netPathPrefixReplace.1	The mapped network directory mount name on the server used to access the run data directory.	/mnt/run_data
miseqdx.seqservice.eventFileDirectorySuffixes	A list of eventFileDirectory path entries to monitor for event files. The value is one or more comma separated integers.	99
miseqdx.seqservice.runReportViewsVersion	The current version of the Run Report views in the database. The value 0 represents the state before the views are created. This property is automatically updated by the run report.	0

Property	Description	Default Value
miseqdx.seqservice.netPathPrefixSearchReplaceSuffixes	A list of netPathPrefix search and replace entries for transforming Windows to Linux network paths. The value is one or more comma-separated integers.	99
miseqdx.seqservice.variantCallingProcessBaseName 'Variant Calling (MiSeqDx)'	Sequencing process type base display name. Partial matching is used to look up the process type.	Variant Calling (MiSeqDx)

Support for multiple seqservice.netPathPrefixSearch property values

It is possible to configure support for multiple, identical seqservice.netPathPrefixSearch property values. For details, see Configuring Multiple Identical netPathPrefixSearch Values.

Reagent categories / label groups installed

The following reagent categories / label groups are included in the default configuration for the Illumina MiSeqDx Integration Package:

- CF 139-Variant Assay Indexes
- CF 139-Variant Assay 8-Sample Indexes
- CF Clinical Sequencing Assay 8-Sample Indexes
- Universal Kit Indexes
- Universal Kit 8-Sample Indexes

Reagent kits installed

The following reagent kits are included in the default configuration for the Illumina MiSeqDx Integration Package:

- CF 139-Variant Assay-Oligo Pool
- CF Clinical Sequencing Assay-Oligo Pool
- Custom Oligo Pool
- Elution Buffer
- EtOH
- Extension-Ligation Mix
- Hybridization Buffer
- Index Primers
- Library Beads
- Library Dilution Buffer
- Library Normalization Diluent
- Library Normalization Wash
- Library Storage Buffer
- MiSeqDx Flow Cell - CF 139-Variant Assay
- MiSeqDx Flow Cell - CF Clinical Sequencing Assay
- MiSeqDx Flow Cell - Universal kit
- MiSeqDx SBS Solution (PR2) - CF 139-Variant Assay
- MiSeqDx SBS Solution (PR2) - CF Clinical Sequencing Assay
- MiSeqDx SBS Solution (PR2) - Universal Kit
- NaOH
- PCR Clean-Up Beads
- PCR Master Mix
- PCR Polymerase
- Stringent Wash Buffer
- Universal Wash Buffer

Controls installed

The following controls are included in the default configuration for the Illumina MiSeqDx Integration Package:

- Negative Control for MiSeqDx
- Positive Control for MiSeqDx

Container types installed

The following container types are included in the default configuration for the Illumina MiSeqDx Integration Package:

- MiSeqDx Flow Cell - Universal Kit
- MiSeqDx Flow Cell - CF 139-Variant Assay
- MiSeqDx Flow Cell - CF Clinical Sequencing Assay
- MiSeqDx Reagent Cartridge - CF 139-Variant Assay
- MiSeqDx Reagent Cartridge - CF Clinical Sequencing Assay
- MiSeqDx Reagent Cartridge - Universal Kit

See also Support for container types.

Instrument integration

Note:

To ensure that your Illumina instrument warranty remains valid, the instrument integration must be performed and maintained by the LIMS support team. To perform this integration, the LIMS support team will require direct access to the instrument via WebEx or Remote Desktop while the instrument is idle.

Below is an overview of the steps performed by the LIMS support team when configuring the instrument for use with the Illumina MiSeqDx Integration to LIMS.

Configuring the Illumina instrument:

1. Create directory on the local computer to hold the batch files. These batch files write event files to the network attached storage (NAS) share(s).
2. Create directory on the NAS to hold the event files.
3. Modify Illumina software configuration files to call the batch files that create the event files.
4. Update sequencing service default database properties to match the specifics of the installation.

Configuring instruments

We recommend configuring sequencer instrument names in LIMS to track the specific lab instrument on which a run was completed.

This information should be configured on the MiSeqDx Run (MiSeqDx) 1.0 step, and should be set to the same name as is configured in the Illumina sequencing software – i.e., this will be value of the Instrument Run ID field described in the Metadata section above.

When the integration reads the Instrument Run ID field in the run results, it will update the instrument on the step in LIMS, eliminating the need for the user to manually enter this information.

For instructions on configuring instrument names, refer to the LIMS Documentation – see *Adding Instruments and Instrument Types* in the System Configuration forum.

Configuring sample sheet generation

Sample sheet generation occurs on the step prior to the sequencing run – Denature, Dilute and Load Sample 1.0 – which is the step where samples are placed on the container that will be loaded in the instrument.

By default, one instrument sample sheet is created for the container loaded during the Denature, Dilute and Load Sample 1.0 step.

Support for container types

All one-dimensional container types with both numeric rows and numeric columns are supported.

Rules and constraints

This integration operates with the following constraints:

- The Reagent Cartridge ID must be unique. There should not be multiple containers in the system with the same name.
- The reagent cartridge ID must be scanned as the Container Name on the Denature, Dilute and Load Sample 1.0 step.
- For MOS sample sheet generation constraints, see Illumina MiSeqDx 1.x Sample Sheet Generation.

Illumina MiSeqDx Integration v1.5 Validation and Troubleshooting

Compatibility: See Release Notes

This guide explains how to use the MiSeqDx Validation workflow that is included in the Illumina MiSeqDx Integration Package. The workflow allows for simple validation of the following functionality:

- Auto-placement of reagent indexes - on PCR Amplification step
- Generation of a sample sheet file for use with the MiSeqDx Operating Software (MOS) - on Denature, Dilute and Load Sample step

In addition, this guide provides tips to help you troubleshoot the system.

All validation steps below assume that you have installed the Illumina MiSeqDx Integration Package and have imported the default LIMS configuration.

Note:
Instructions for configuring sample sheet generation to meet the needs of your lab are provided in the Illumina MiSeqDx User and Configuration Guide.

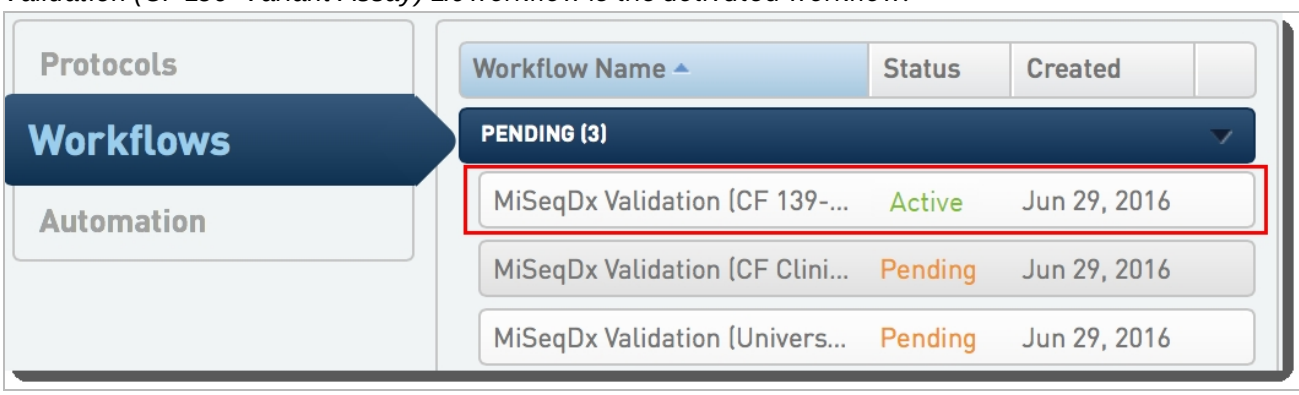
Validating auto-placement of indexes and sample sheet generation

After installing and configuring the integration package, and configuring sample sheet generation (see User and Configuration Guide), follow the steps below to activate the MiSeqDx Validation workflow and run samples through it.

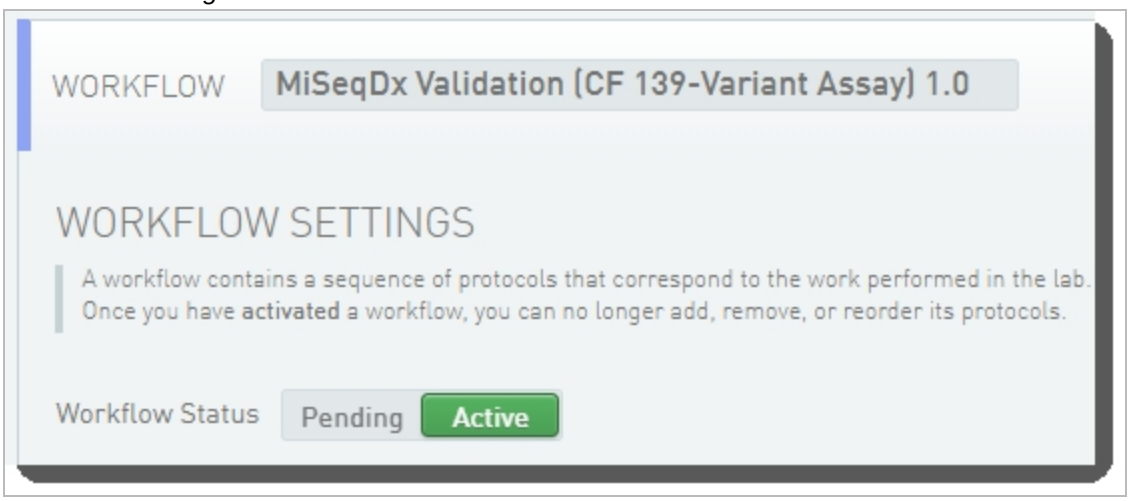
Auto-placement of indexes validation occurs in step 4 ; sample sheet generation validation occurs in step 7.

Validation steps

1. In the the LIMS web interface, on the Configuration > Workflows screen, activate one of the three Validation workflows:
    - MiSeqDx Validation (CF 139-Variant Assay) 1.0
    - MiSeqDx Validation (CF Clinical Sequencing Assay) 1.0
    - MiSeqDx Validation (Universal Kit) 1.0 *Note that in the following screenshots, the MiSeqDx Validation (CF 139-Variant Assay) 1.0workflow is the activated workflow.*



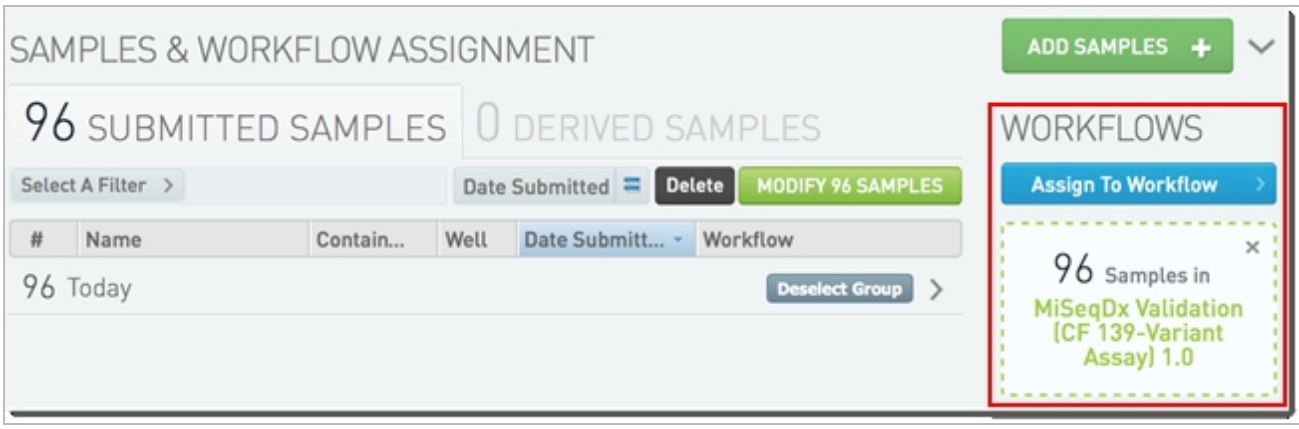
Workflow configuration and activation - LIMS v4.x



Workflow configuration and activation - LIMS v5.x The protocol included in the activated MiSeqDx Validation workflow will display in Lab View. (In this example, this is the MiSeqDx Validation (CF 139-Variant Assay) 1.0 protocol.)

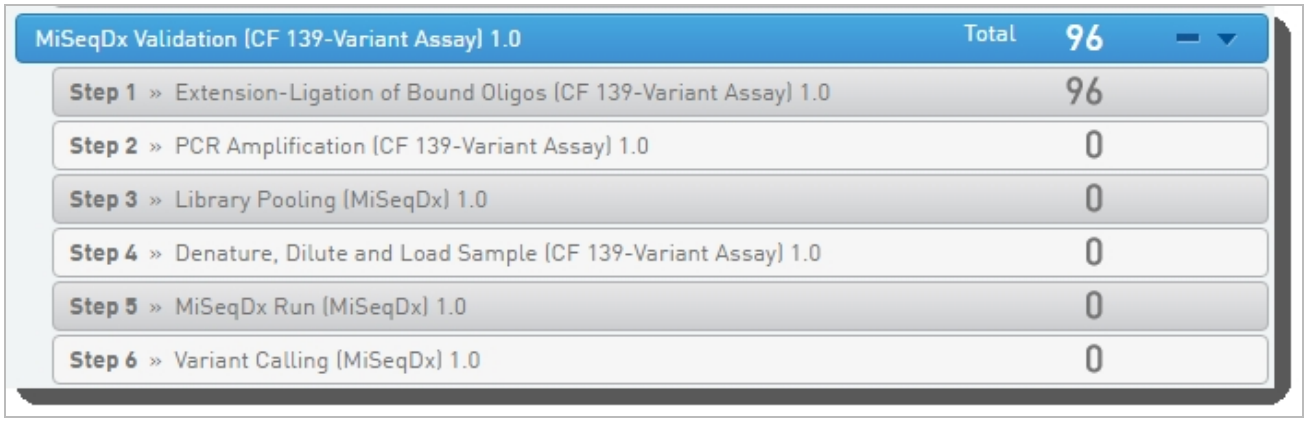


2. On the Projects and Samples screen:
   - Create a test project and add samples to it. The project must contain between 9 and 96 samples.
   - Assign your samples to the MiSeqDx Validationworkflow you activated in step 1.

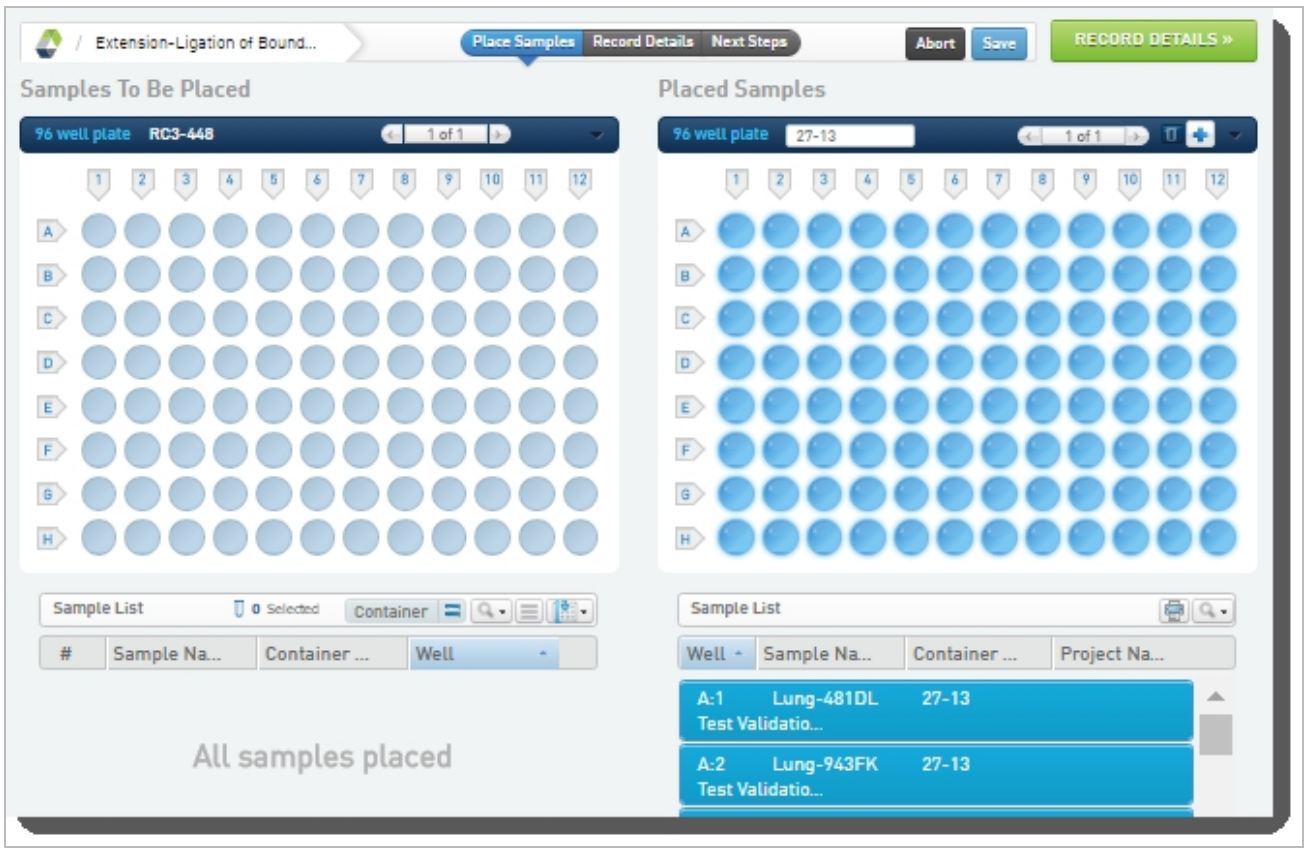


For Research Use Only. Not for use in diagnostic procedures.

3. In Lab View, locate the MiSeqDx Validation protocol. You will see your samples queued for the Extension-Ligation of Bound Oligosstep.



- Add the samples to the Ice Bucket and begin work.
- On the Placement screen, place the samples into the output container. Click Record Details.



For Research Use Only. Not for use in diagnostic procedures.

- On the Record Details screen, under Reagent Lot Tracking, select the Extension-Ligation Mix reagent lot used in the step (you may need to activate the lot on the Reagents / Reagents and Controlsscreen).

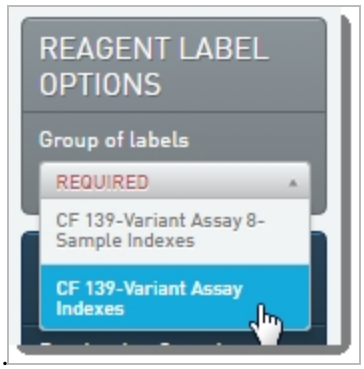


- Click Next Steps.
- On the Assign Next Steps screen. Assign the samples to the PCR Amplificationstep.

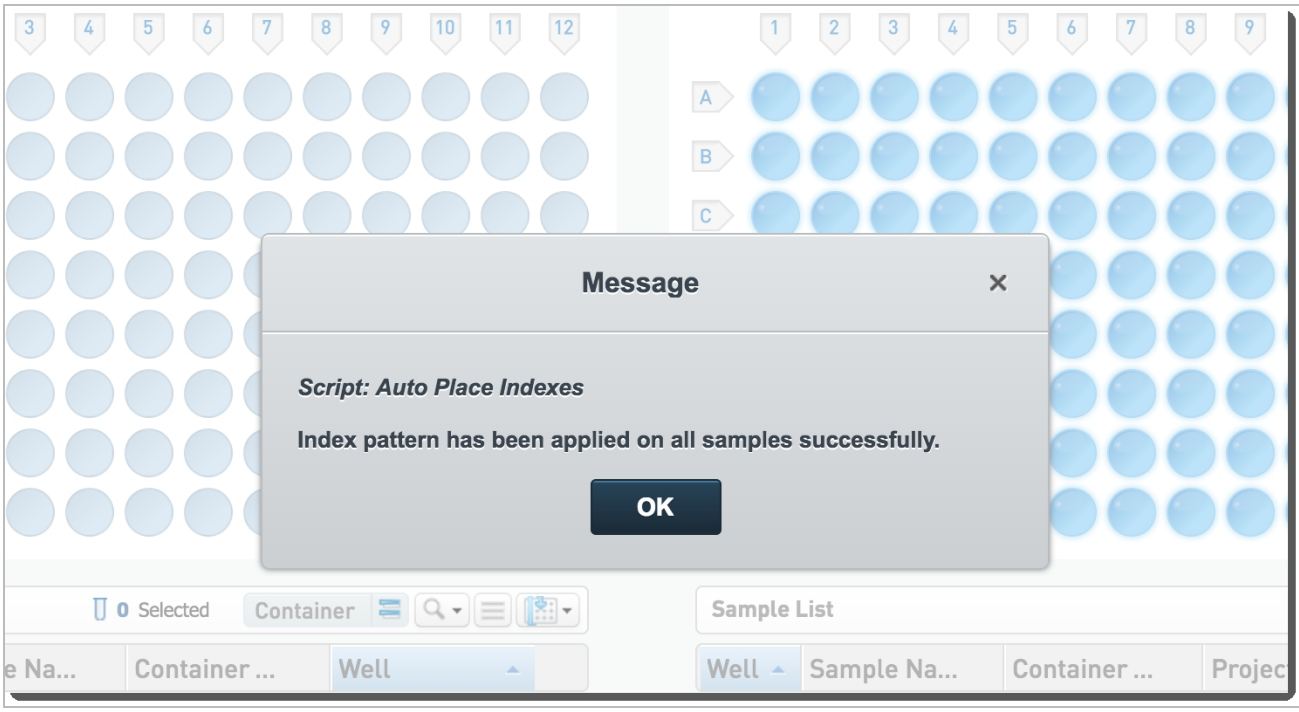


- Click Finish Step.

4. In Lab View, locate the MiSeqDx Validation protocol. You will see your samples queued for the PCR Amplification step.
    - Add the samples to the Ice Bucket.
    - In the Reagent Label Options panel, in the Group of labels list, select CF 139-Variant Assay Indexes(or the equivalent for the workflow you activated).

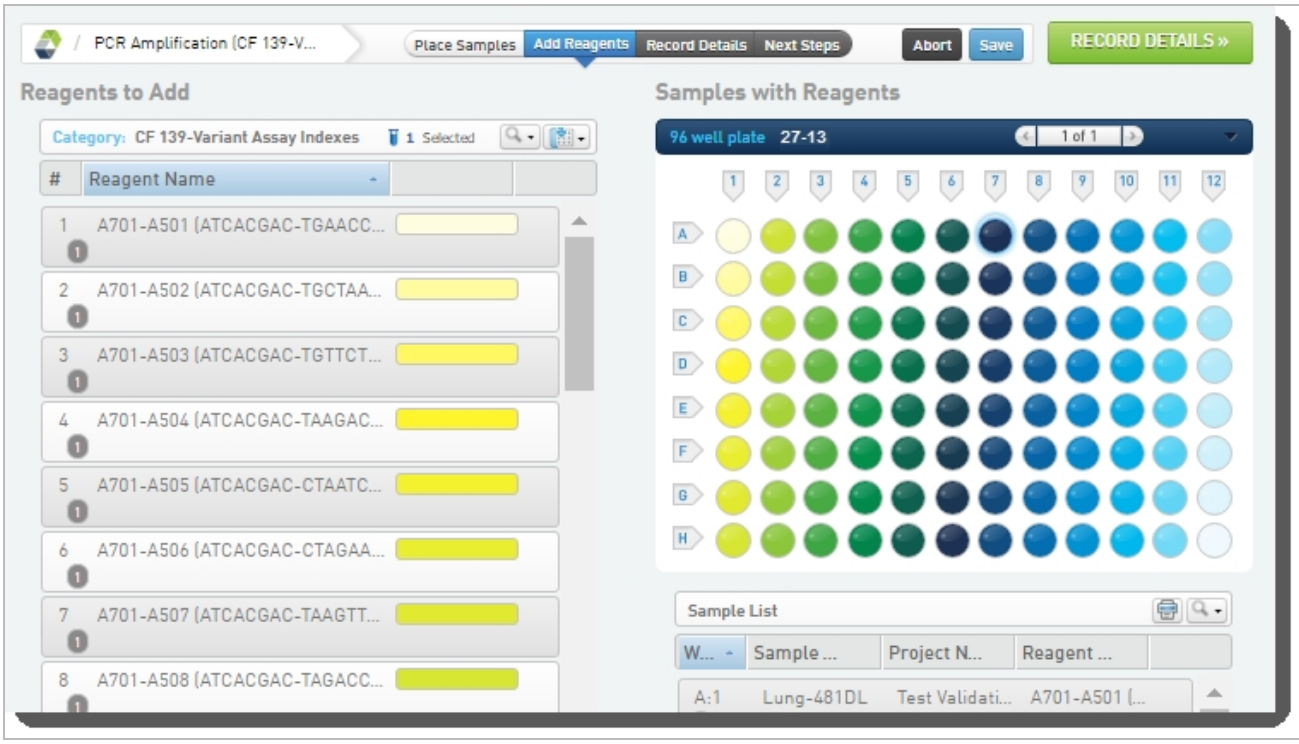


- Click Begin Work.
- On the Placement screen, place the samples into the output container. Click Add Reagents.
- On entry to the Add Reagents screen, the Auto Place Indexes automation is automatically invoked and the reagents are placed on the samples.
- Once placement is complete, a message displays, indicating that the index pattern has been applied successfully. Click OK.

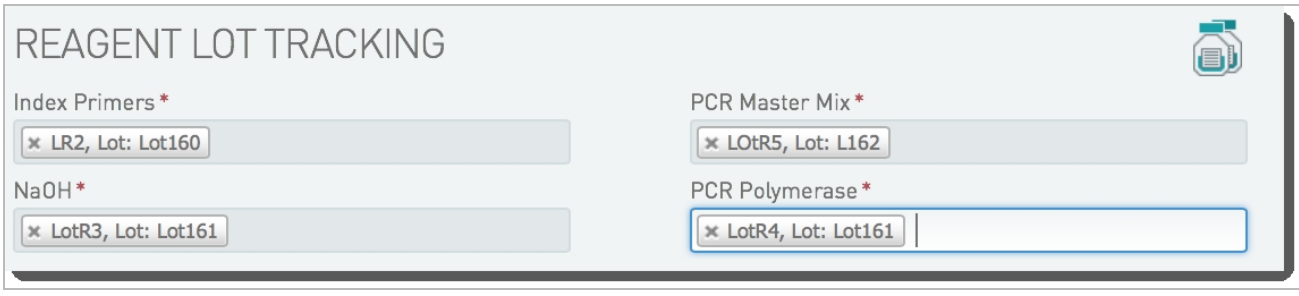


For Research Use Only. Not for use in diagnostic procedures.

- On Add Reagents screen, reagents are already placed. Click Record Details.



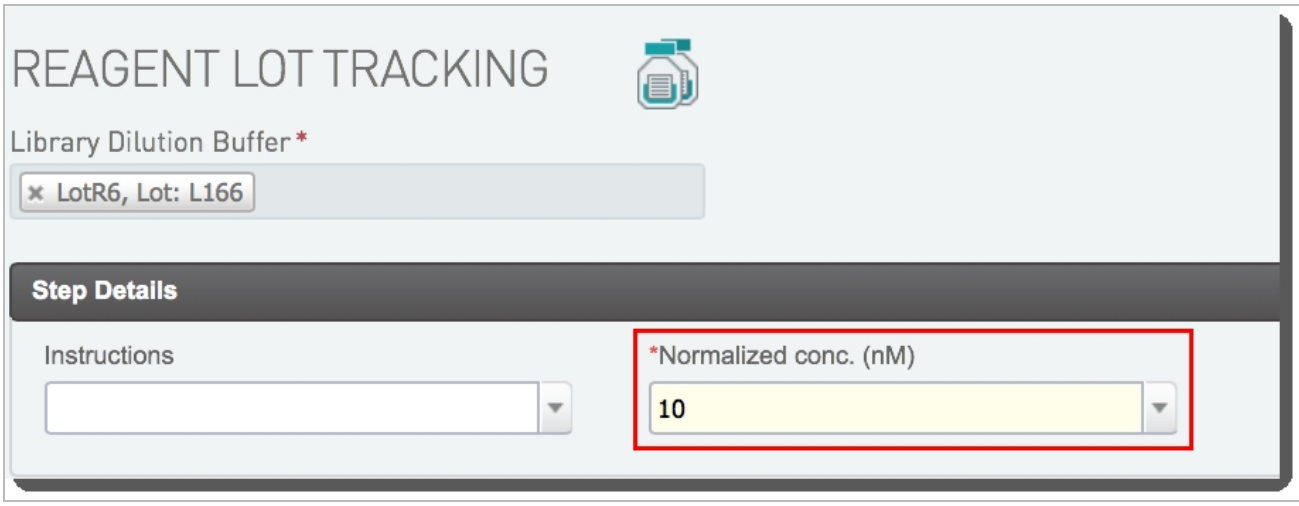
- On exit from the Add Reagents screen, the Validate Index Placement automation is automatically invoked and index placement is validated. Once validation is complete, a message displays, indicating that the index pattern has been validated on all samples successfully. Click OK.
- On the Record Details screen, under Reagent Lot Tracking, select the lots for Index Primers, NaOH, PCR Master Mix, and PCR Polymerase.



- Click Next Steps.
- On the Assign Next Steps screen, assign samples to the Library Pooling (MiSeqDx) 1.0 step. Click Finish Step.

5. Return to Lab View and locate the MiSeqDx Validation protocol. You will see your samples queued for the Library Pooling (MiSeqDx) 1.0 step. Under Reagent Lot Tracking, select the reagent lot used in the step.

- Under Step Details, enter the concentration value in the Normalized conc. (nM)field.

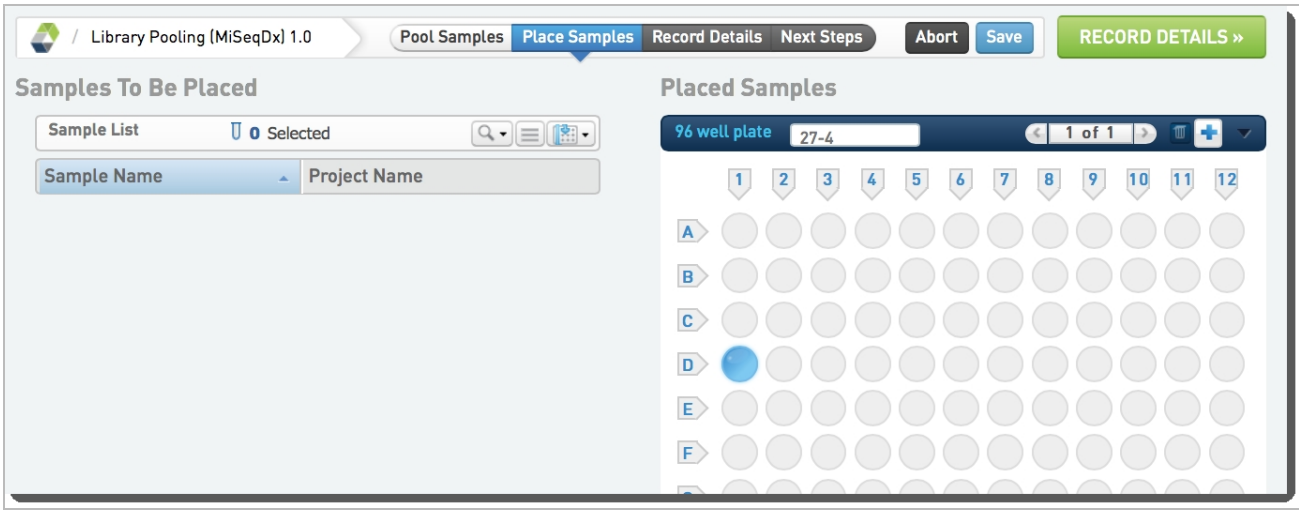


- Click Next Steps.
- Add the samples to the Ice Bucket and begin work.
- In the Add Control Samples panel, select PhiX Internal Control. Click Begin Work.



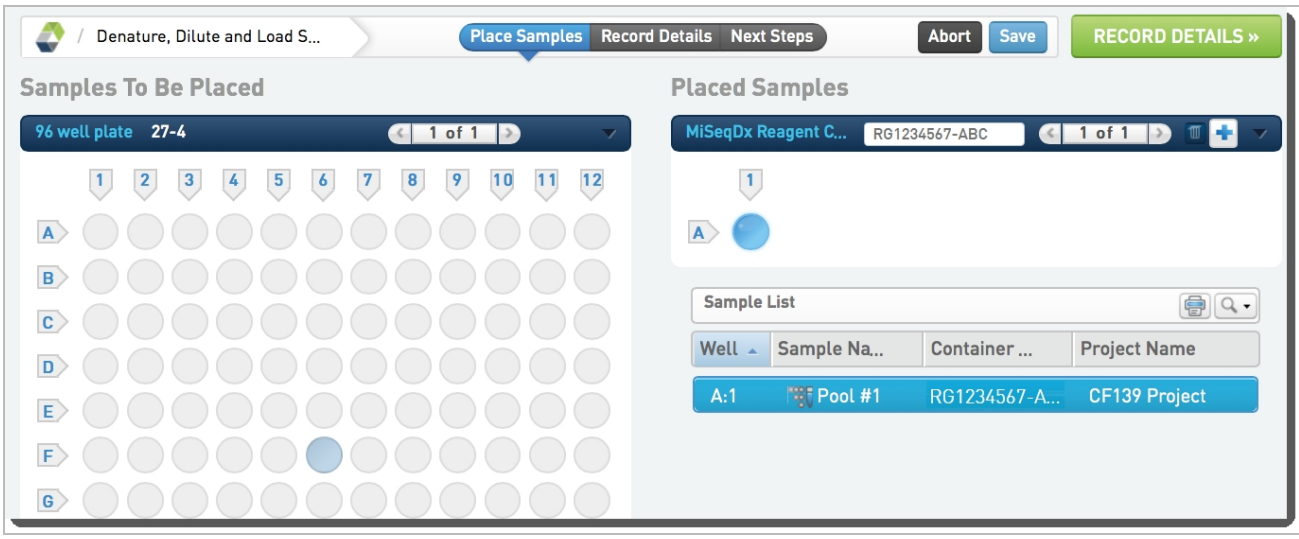
- On the Pool Samples screen, create a pool of samples. Click Place Samples.

- On the Placement screen, place the pool into the output container. Click Record Details.



- On the Record Details screen, click Next Steps.
- On the Assign Next Steps screen, assign the pool to the Denature, Dilute and load Samples step.

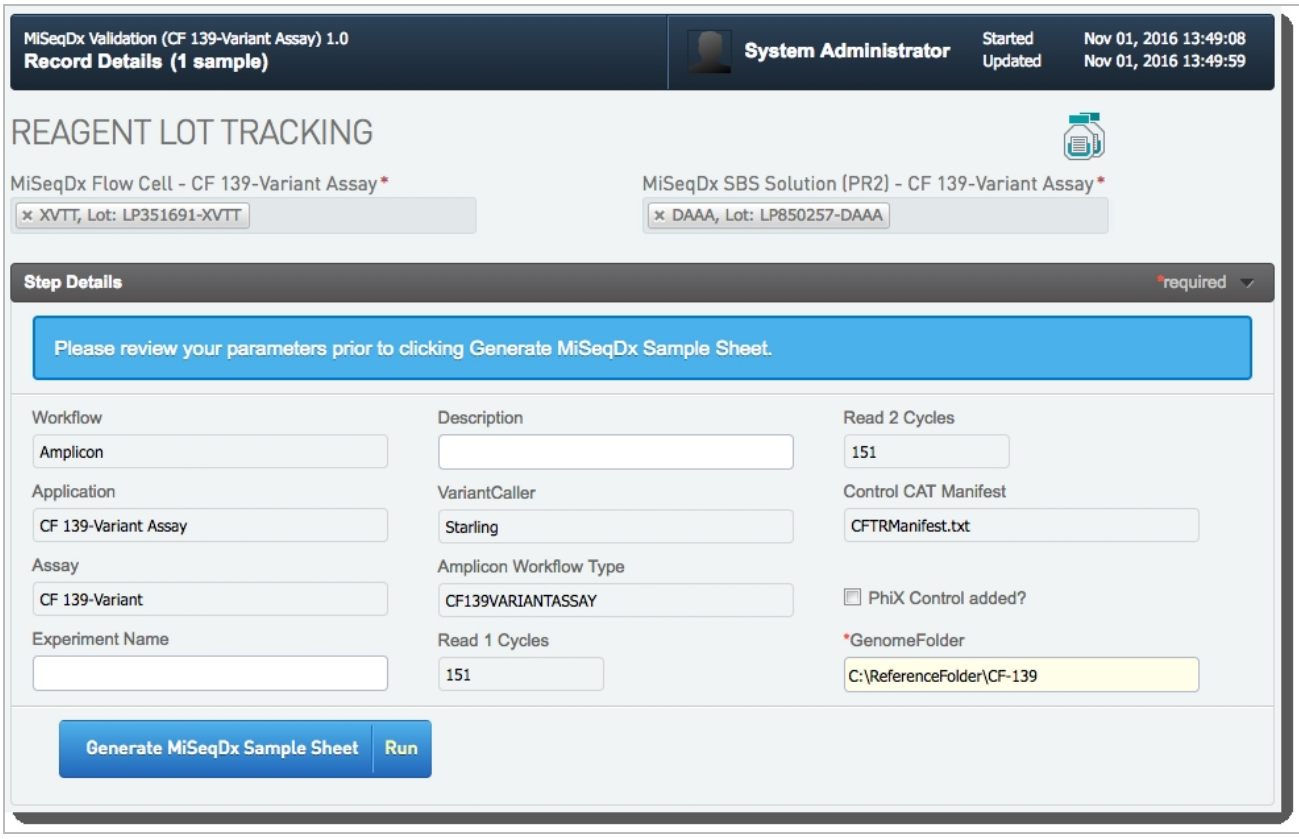
6. Return to Lab View and locate the MiSeqDx Validation protocol. You will see your pooled samples queued for the Denature, Dilute and Load Sample step.
   - Add the pool to the Ice Bucket and begin work.
   - On the Placement screen, place the pool into a MiSeqDx Reagent Cartridge.



   - Click Record Details. On entry to the Record Details screen, a script validates the Container Name and prompts you to scan the Reagent Cartridge barcode.

7. On the Record Details screen of the Denature, Dilute and Load Sample step:
   - Under Reagent Lot Tracking, select the reagent lots used in the step.

- Click Generate MiSeqDx Sample Sheetto generate the MiSeqDx sample sheet.

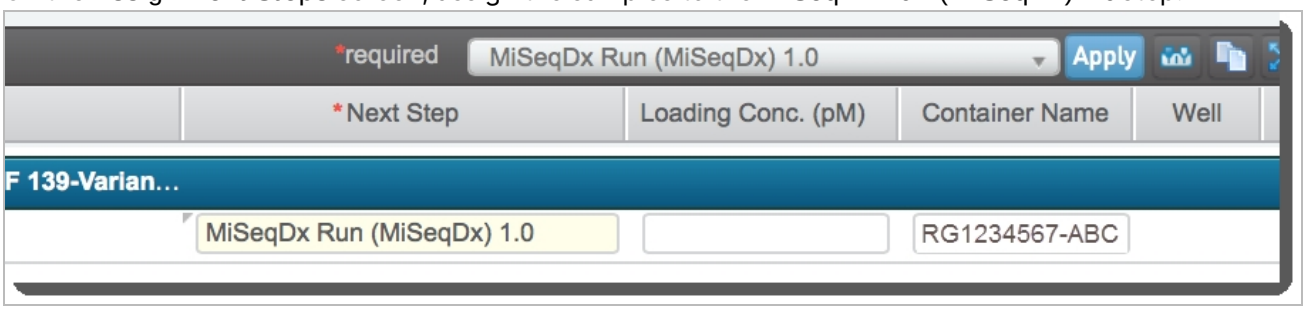


- The LIMS attaches the generated sample sheet and log file to placeholders in the Files area of the Record Detailsscreen. Download the files and validate their format and content.



- Click Next Steps.

8. On the Assign Next Steps screen, assign the samples to the MiSeqDx Run (MiSeqDx) 1.0step.



Validating creation of event files

Follow the steps below to confirm that event files are created by the batch file in the destination path. The steps assume that the default configuration has been successfully imported.

This test validates that:

- Your DESTINATION_PATH is correctly configured.
- The instrument computer can access and write to the DESTINATION_PATH.
- There are no syntax errors in the LIMS batch file.

Steps

1. In C:\gls, double-click gls_event_mos_rta.bat
2. Confirm that an 'empty' event file appears in the configured DESTINATION_PATH.
3. Manual invocation of gls_event_mos.bat will produce output resembling the example EndRun event file attached below.

Troubleshooting

If an automation trigger does not appear to run its corresponding scripts, see:

- *Troubleshooting Automated Informatics* in the LIMS documentation (Automated Informatics section)
- *Troubleshooting EPP / Automation* in the API > Overview > API Advanced Topics section

If an error occurs that does not provide direction on how to proceed:

1. Confirm the version of the installed Illumina MiSeqDx Integration Package by running the following command from the server console:

```
rpm -qa | grep -i miseqdx
```

2. If the error is related to retrieving run results data, review the MiseqDxIntegrator.logContact the BaseSpace Clarity LIMS Support team, supplying the relevant information from the troubleshooting already performed.
   page.

Illumina MiSeqDx v1.5 Release Notes

- MiSeq Operating Software (MOS) v1.1.7
- Real-Time Analysis (RTA) v1.16.18
- MiSeq Reporter Software (MRS) v2.2.31.1

Features to note for this release

The Illumina MiSeqDx Integration Package v1.5 includes support for both MiSeqDx Research mode and Diagnostic mode.

- For Diagnostic mode, this integration package supports the following three kits: CF 139-Variant Assay, CF Clinical Sequencing Assay, and Universal Kit.
- For Research mode, the Illumina MiSeq Integration Package v5.3 is also included.

New features

This release supports the integration between BaseSpace Clarity LIMS and the MiSeqDx instrument. At a high level, this integration includes the following features:

- In CF 139-Variant Assay and Universal Kit workflows, indexes are automatically placed on samples when running the PCR Amplification step in the Library Prep protocol.
- Key metrics are parsed back into LIMS following the completion of primary analysis on the instrument.
- Variant call files are attached to the Variant Calling step in the LIMS, individually for each sample and one combined file for all the samples together.

Defects fixed

None

Known issues

This release contains the following known issues, which are prioritized for a later release:

- Following a sequencing run, user must wait until the run report is automatically generated and attached before finishing the MiSeqDx Run step. If user completes the step before these events occur, they cannot return to the step and regenerate the report.
- The metrics parsed back into the LIMS for %Bases Q30 R1 and R2 are not consistent with Sequencing Analysis Viewer.

Documentation

For detailed information related to this release, see the following documentation:

- Illumina MiSeqDx Integration v1.5 Validation and Troubleshooting Guide
- Illumina MiSeqDx Integration v1.5 Installation Guideline
- Illumina MiSeqDx Integration v1.5 User and Configuration Guide
- Illumina MiSeqDx Sample Sheet Generation

Illumina MiSeqDx v1.1 Release Notes

Release Date: August 12, 2016

Version: 1.1

Compatibility: BaseSpace Clarity LIMS v4.1 or later; NGS Extensions Package v5.10 or later

Illumina MiSeqDx Software Compatibility:

- MiSeq Operating Software (MOS) v1.1.7
- MiSeq Reporter Software (MRS) v2.2.31.1

Features to note for this release

New features

- Functionality to track positive and/or negative controls in a sequencing run.
- Updated reagent categories for CF Clinical, CF 139-Variant, and Universal Kit.

Defects fixed

- Updated bcl2fastq2 sample sheet generation documentation to show the correct field and entity information.

Known limitations

None

Known issues

None

Documentation

For detailed information related to this release, see the following documentation:

- Illumina MiSeqDx Validation and Troubleshooting Guide
- Illumina MiSeqDx Internal Installation Guide
- Bcl2fastq2 Sample Sheet Generation

Illumina MiSeqDx v1.0 Release Notes

Release Date: June 30, 2016

Version: 1.0

Compatibility: BaseSpace Clarity LIMS v4.1 or later; NGS Extensions Package v5.10 or later

Illumina MiSeqDx Software Compatibility:

- MiSeq Operating Software (MOS) v1.1.7
- MiSeq Reporter Software (MRS) v2.2.31.1

Features to note for this release

New features

- Functionality to generate sample sheet for CF Clinical Sequencing Assay, CF 139-Variant Assay, and Universal Kit.
- Functionality to support Diagnostic mode only.

- PhiX control may be added to the run and sample sheet can be generated.
- Automation to support bcl2fastq v2.17 sample sheet generation for downstream analysis.

Defects fixed

Not applicable

Known limitations

The following are known limitations. These are prioritized for a future release:

- Sample sheet generation does not support including positive and/or negative controls in a sequencing run.

Known issues

The following are known defects. These are prioritized for a future release:

- The documentation for bcl2fastq2 sample sheet generation contains some inconsistent column and incorrect entity information.

Documentation

For detailed information related to this release, see the following documentation:

- Illumina MiSeqDx Validation and Troubleshooting Guide

HiSeq Integrations

The Illumina HiSeq instrument integration provides preconfigured workflows that map to established lab protocols and steps used with HiSeq instruments. The integration also supports the associated library prep kits, reagent kits, and assays used in these protocols.

Illumina HiSeq/GAIIx v5.5.2 Release Notes

Last Modified: August 18, 2016

Release date: August 18, 2016

Version: HiSeq 5.5.2

Compatibility: BaseSpace Clarity LIMS v4.0 or later, with NGS Extensions Package v5.10 or later

Illumina HiSeq Software Compatibility:

- HiSeq Control Software (HCS) v2.2.7
- Real Time Analysis (RTA) Software v1.18.64

Features to Note for this Release

New functionality

- Automation to support bcl2fastq v2.17 sample sheet generation for downstream analysis.

Defects Fixed

- Fixed an issue to prevent the sequencing service from restarting continuously if file permissions are incorrect.
- Increased security to protect against a known Java deserialization vulnerability on the API utilities component.
- Updated bcl2fastq2 sample sheet generation documentation to show the correct field and entity information.
- Fixed an issue to display the correct value in the LIMS for Intensity Cycle 1 of Read 2 after a sequencing run.
- Fixed an issue to handle API requests for queries that are exceedingly long.

Known Issues

This release contains the following known issues, which are prioritized for a later release:

- The Run Report may display Rapid Run Flow Cell data incorrectly, since it currently calculates based on an 8 lane flow cell.

Documentation

For detailed information related to this release, see the following documentation:

- *Illumina HiSeq/GAIIx Integration v5.5 / v5.6 Configuration and User Interaction* on page 2333
- *Illumina HiSeq/GAIIx Integration v5.5 / v5.6 Validation and Troubleshooting* on page 2362
- *CASAVA 1.8.2 Sample Sheet Generation*
- *Bcl2fastq2 Sample Sheet Generation (NGS v5.16 and earlier)*

Illumina HiSeq/GAIIx v5.5.1 Release Notes

Last Modified: June 1, 2016

Release date: May 31, 2016

Version: HiSeq 5.5.1

Compatibility: BaseSpace Clarity LIMS v4.0 or later, with NGS Extensions Package v5.10 or later

Illumina HiSeq Software Compatibility:

- HiSeq Control Software (HCS) v2.2.7
- Real Time Analysis (RTA) Software v1.18.64

Features to Note for this Release

New functionality

No new functionality in this release.

Defects fixed

- Fixed an issue to successfully parse unbinned QMetrics data upon sequencing run completion.
- Increased security to protect against a known Java deserialization vulnerability.

Known Issues

This release contains the following known issues, which are prioritized for a later release:

- The Run Report may display Rapid Run Flow Cell data incorrectly, since it currently calculates based on an 8 lane flow cell.
- The documentation for bcl2fastq2 sample sheet generation contains some inconsistent column and incorrect entity information.

Documentation

For detailed information related to this release, see the following documentation:

- *Illumina HiSeq/GAIIx Integration v5.5 / v5.6 Configuration and User Interaction* on page 2333
- *Illumina HiSeq/GAIIx Integration v5.5 / v5.6 Validation and Troubleshooting* on page 2362

- *CASAVA 1.8.2 Sample Sheet Generation*
- *Bcl2fastq2 Sample Sheet Generation (NGS v5.16 and earlier)*

Illumina HiSeq/GAIIx Release Notes (for Clarity LIMS 4.0 or later)

Release date: March 29, 2016

Version: HiSeq 5.5

Compatibility: BaseSpace Clarity LIMS v4.0 or later, with NGS Extensions Package v5.10 or later

Illumina HiSeq Software Compatibility:

- HiSeq Control Software (HCS) v2.2.7
- Real Time Analysis (RTA) Software v1.18.64

Features to Note for this Release

New Functionality

- Automation to support either CASAVA 1.8.2 or bcl2fastq2 sample sheet generation for downstream analysis.
- Increased security to protect against a known Java deserialization vulnerability.
- Ability to allow single and dual indexes on different lanes on the same flow cell, and allow different lengths of single indexes in different lanes on the same flow cell.
- Improved HiSeq documentation (*Illumina HiSeq/GAIIx Integration v5.5 / v5.6 Configuration and User Interaction* on page 2333 and *Illumina HiSeq/GAIIx Integration v5.5 / v5.6 Validation and Troubleshooting* on page 2362 - see section below).

Defects Fixed

- Addressed an issue where the Finish Date was not correctly populated with the actual EndRun event date.
- Clarified messaging for when there is improper data related to well placement of a sample.
- Fixed the column width for the Clusters Raw summary value on the Run Report to support larger values.
- This release contains the following known issues, which are prioritized for a later release:
- Fixed an issue in the bcl2fastq2 sample sheet for pooling of non-indexed samples. The Sample Name column will now contain Pool Name; the SampleID column will now contain Pool LIMSID.
- Fixed an issue in the CASAVA sample sheet for pooling of non-indexed samples. The Sample Name column will now contain Pool Name; the SampleID column will now contain Pool LIMSID.

Known Issues

This release contains the following known issues, which are prioritized for a later release:

- The Run Report may display Rapid Run Flow Cell data incorrectly, since it currently calculates based on an 8 lane flow cell.
- Upon sequencing run completion, there is an issue in parsing unbinned QMetrics data values.

Documentation

For detailed information related to this release, see the following documentation:

- *Illumina HiSeq/GAIIx Integration v5.5 / v5.6 Configuration and User Interaction* on page 2333
- *Illumina HiSeq/GAIIx Integration v5.5 / v5.6 Validation and Troubleshooting* on page 2362
- *CASAVA 1.8.2 Sample Sheet Generation*
- *Bcl Conversion for CASAVA*

Illumina HiSeq/GAIIx Integration v5.5 / v5.6 Configuration and User Interaction

Compatibility: See *Illumina HiSeq/GAIIx Release Notes (for Clarity LIMS 4.0 or later)* on page 2332.

Contents

The Illumina HiSeq/GAIIx Integration Package v5.5 supports Illumina HiSeq 1000, 1500, 2000, 2500, GAII(x), and HiScanSQ instruments.

The integration provides automated generation of a sample sheet file for use with the HiSeq or GAII(x) sequencing control software (HCS/SCS) and with bcl2fastq v2.15 or CASAVA v1.8.2 analysis software, and allows for automated tracking of an Illumina sequencing run in BaseSpace Clarity LIMS. Tracking includes instrument run status, automated reporting, and capturing and parsing of run statistics.

- This page describes the integration between Clarity LIMS and the IlluminaHiSeq/GAII(x) system. It includes information about protocols and automations, script parameters and usage, configuration options, installed components, and rules and constraints.
- For instructions on validating and troubleshooting the Illumina HiSeq/GAIIx Integration Package v5.5, see the *Illumina HiSeq/GAIIx Integration v5.5 / v5.6 Validation and Troubleshooting* on page 2362.
- For overview information about how the Illumina – Clarity LIMS interface works, and which events are monitored, see *How the Illumina Instrument and LIMS Software Interact*.

Overview

This Illumina HiSeq/GAIIx Integration includes the following:

- Preconfigured TruSeq Exome Enrichment 5.0, Illumina SBS (HiSeq GAIIx) 5.0, and HiSeq/GAIIx Validation protocols

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- Automatic generation of sample sheet files for use with bcl2fastq v2.15 or CASAVA v1.8.2 analysis software
- Automated tracking in Clarity LIMS of the progress and metrics of an Illumina sequencing run
- Per-instrument tracking of sequencing runs
- Ability to directly monitor run status (cycle number) across multiple instruments from within Clarity LIMS
- Automated tracking of Real Time Analysis (RTA) run directory location and other run specific information
- Automated tracking of sequencing run parameters
- Automatic generation of a report, which includes the current run's statistics and a comparison to the last five runs for any statistics that are one standard deviation off
- Integration with Illumina's Bcl to FASTQ conversion and demultiplexing tool for CASAVA

Information on these features is provided in the following sections.

Protocols and Automations

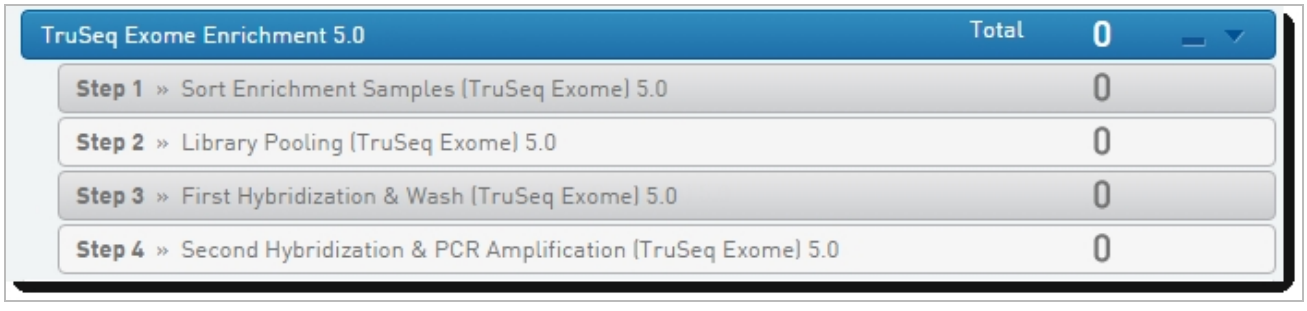
The following out-of-the-box protocols are included in the Illumina HiSeq/GAIIx Integration Package v5.5:

- TruSeq Exome Enrichment 5.0
- Illumina SBS (HiSeq GAIIx) 5.0
- HiSeq/GAIIx Validation

Figures 1 - 3 show these protocols as they display to the lab scientist working in Platform SW Clarity LIMS Lab View.

TruSeq Exome Enrichment 5.0 Protocol

Figure 34 Figure 1: Platform SW Clarity LIMS Lab View showing out-of-the-box TruSeq Exome Enrichment 5.0 protocol and steps



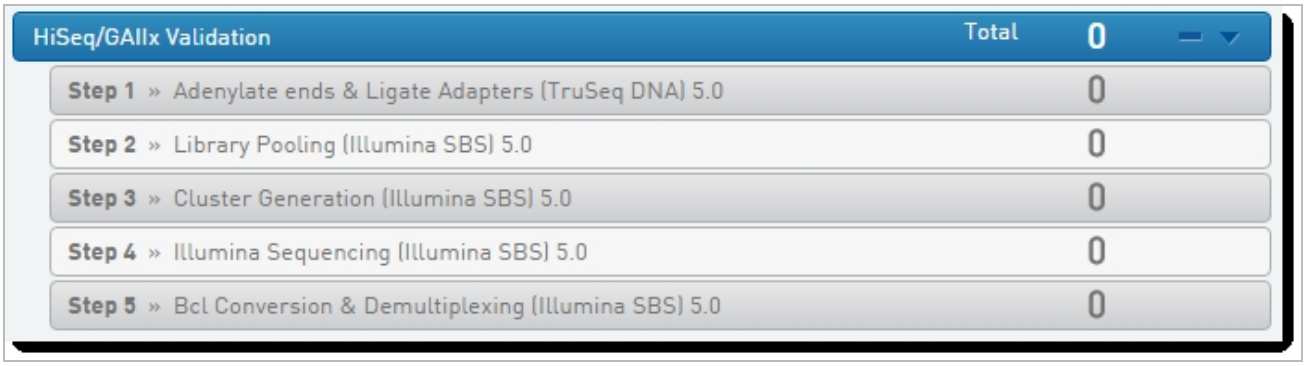
Illumina SBS (HiSeq GAIIx) 5.0 Protocol

Figure 35 Figure 2: Platform SW Clarity LIMS Lab View showing out-of-the-box Illumina SBS (HiSeq GAIIx) 5.0 protocol and steps



HiSeq/GAIIx Validation Protocol

Figure 36 Figure 3: BaseSpace Clarity LIMS Lab View showing HiSeq/GAIIx Validation protocol and step



For instructions on using the HiSeq/GAIIx Validation workflow to validate automated sample sheet generation, see the *Illumina HiSeq/GAIIx Integration v5.5 / v5.6 Validation and Troubleshooting* on page 2362.

Automations

Table 1 lists the automations included in this integration, and the protocol steps on which they are configured. Additional details are provided in the sections following the table.

Note that the TruSeq Exome Enrichment 5.0 protocol does not include any automation.

Figure 37 Table 1: Illumina HiSeq/GAIIx Integration out-of-the-box protocols and automations

Protocol	Automation and step
Illumina SBS (HiSeq GAIIx) 5.0	• Create Normalization CSV – *Configured in Library Normalization (Illumina SBS) 5.0 step* • Assign QC flags – *Configured in qPCR QC 5.0step*

Protocol	Automation and step
Illumina SBS (HiSeq GAIIx) 5.0 HiSeq/GAIIx Validation	1. Generate HiSeq Sample Sheet 2. Generate CASAVA Sample Sheet 3. Generate bcl2fastqv2 Sample Sheet - *Configured in Cluster Generation (Illumina SBS) 5.0 step* One of these automations is disabled upon installation of the integration. See Configuring Sample Sheet Generation. • Bcl conversion and demultiplexing – *Configured on Bcl Conversion and Demultiplexing (Illumina SBS) 5.0 step* Depending on the requirements of the system, the BCL Conversion and Demultiplexing (Illumina SBS) 5.0 step may be removed from the protocol. This is typically the case when the system is configured to use the bcl2fastq2 sample sheet for downstream analysis. See Configuring Sample Sheet Generation.

The following sections describe how these automations work, and how the lab scientist interacts with them in the lab.

For more information on automations, including how to configure them, see the following topics in the LIMS Documentation.

LIMS v4.1 and v4.2 Documentation:

- *Configuring Protocol Steps* (Protocols section)
- *Passing Parameters via EPP* (Integrations via EPP section)

LIMS v5 Documentation:

- *Configuring Protocol Steps* (Protocols section)
- *Passing Files and Tokens to Third-Party Programs* (Automations section)

As of Platform SW Clarity LIMS v5.0, the term user-defined field (UDF) has been replaced with custom field in the user interface (the API resource is still called udf).

There are two types of custom fields:

- Master step fields - configured on master steps. These fields only apply to the master step on which they are configured, and the steps derived from those master steps.
- Global fields - configured on entities such as submitted sample, derived sample, measurement, etc. These fields apply to the whole Clarity LIMS system.

Library Normalization File Generation

How it Works

See Normalization Buffer Volumes.

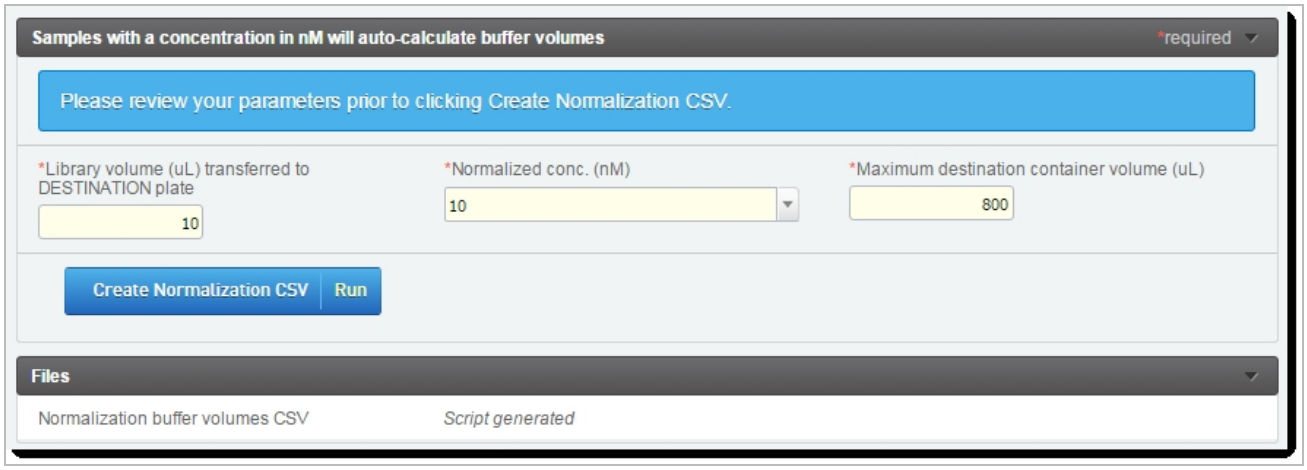
For Research Use Only. Not for use in diagnostic procedures.

User Interaction

On the Record Details screen of the Library Normalization (Illumina SBS) 5.0 step:

1. Enter values for each of the following:
   - Library volume (uL) transferred to destination plate
   - Normalized conc (nM)
   - Maximum destination container volume (uL)
   - Any custom fields/UDFs
2. Click Create Normalization CSV.

Figure 38 Figure 4: Record Details screen of Library Normalization (Illumina SBS) 5.0 step



- Once the script has completed, the Normalization buffer volumes CSV file is generated and attached to the step.

QC Flag Assignment

How it Works

The assignQC script compares the values entered into the Sample Details table by the lab scientist with the criteria values set on the Record Details screen, and uses this information to set appropriate pass or fail QC flags on the output samples.

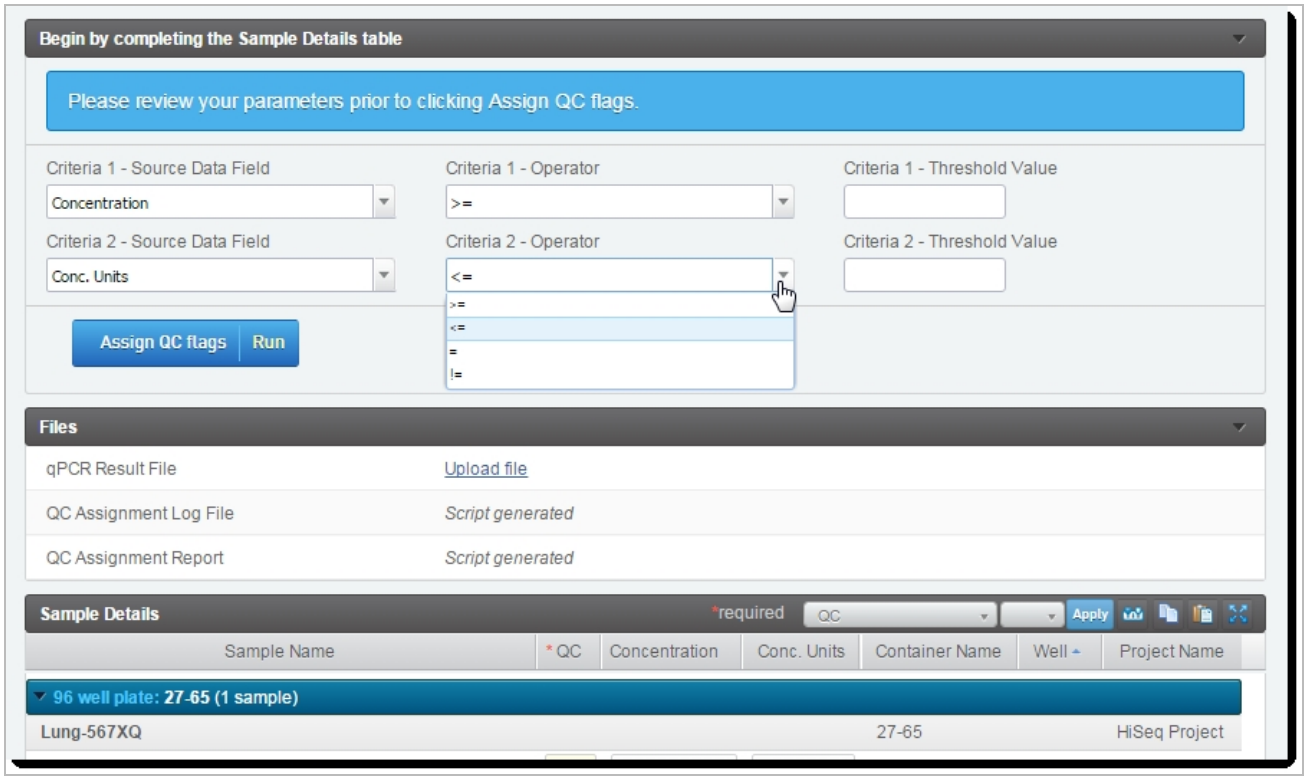
User Interaction

On the Record Details screen of the qPCR QC 5.0 step:

1. Upload the qPCR Result File to the placeholder and manually complete the Sample Details table.
2. Set the desired Criteria Source Data Fields, Criteria Operators, and Criteria Threshold Values.
3. Click Assign QC flags.

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Figure 39 Figure 5: Record Details screen of qPCR QC 5.0 step



4. Once the script has completed:
    - QC flags are set on the output samples
    - The QC Assignment Log File and QC Assignment Report are generated and attached to the step

Sample Sheet Generation

The integration allows for automatic generation of two sample sheets:

- One sample sheet designed to be used with the instrument control software
- One sample sheet designed to be used with CASAVA v1.8.2 or bcl2fastq v2.15 sequencing analysis software. One of these analysis options is disabled when the system is initially configured – see *Configuring Sample Sheet Generation* on page 2354.

By default, one instrument sample sheet and one analysis sample sheet are created for the flow cell loaded during the Cluster Generation (Illumina SBS) 5.0 step.

If required, you may configure the step to generate multiple analysis sample sheets, and to generate sample sheets for multiple flow cells.

Performing analysis with CASAVA or bcl2fastq2 software is optional. If you prefer to use a different analysis pipeline, you will need to create your own sample sheet. You can use Illumina Experiment Manager to do this.

Quick Links:

- Guided sample sheet creation and setup
- Illumina Experiment Manager User Guide
- Illumina Experiment Manager Support

Figure 40 Table 2: Sample sheet files and placeholders generated by Cluster Generation (Illumina SBS) 5.0 step

Item	Description
Lab Tracking Form placeholder	This placeholder in the LIMS allows the user to manually attach a lab-specific tracking form to the step.
HiSeq Sample Sheet (CASAVA format)	This CSV file is automatically generated by the LIMS for use with the HiSeq/GAII(x) instrument. • May be opened as a text file, or as an MS Excel spreadsheet • A copy of the generated sample sheet is automatically placed in the Basecalls folder, the default location required by CASAVA
Sample Sheet Generation Log	Automatically generated by the LIMS, this log file captures any errors that the LIMS may encounter when generating the HiSeq/GAIIx sample sheet.
Analysis Sample Sheet (for use with CASAVA or bcl2fastq2)	This CSV file is automatically generated by the LIMS for use in CASAVA or bcl2fastq2 post-sequencing analysis. • May be opened as a text file, or as an MS Excel spreadsheet • If the CASAVA sample sheet is in use, a copy of the generated sample sheet is automatically placed in the Basecalls folder, the default location required by CASAVA
Analysis Sample Sheet Generation Log	Automatically generated by the LIMS, this log file captures any errors that the LIMS may encounter when generating the analysis sample sheet.

How it Works

1. Sample sheet generation is configured on the Record Details screen of the Cluster Generation (Illumina SBS) 5.0 step – this is when the samples are loaded onto the flow cell that will be placed in the instrument.
   Figure 6 and Figure 7 show the three automations configured on the step, in LIMS v4.x and LIMS v5.x.

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Figure 41 Figure 6: Cluster Generation *(Illumina SBS) 5.0* automation configuration - LIMS v4.x

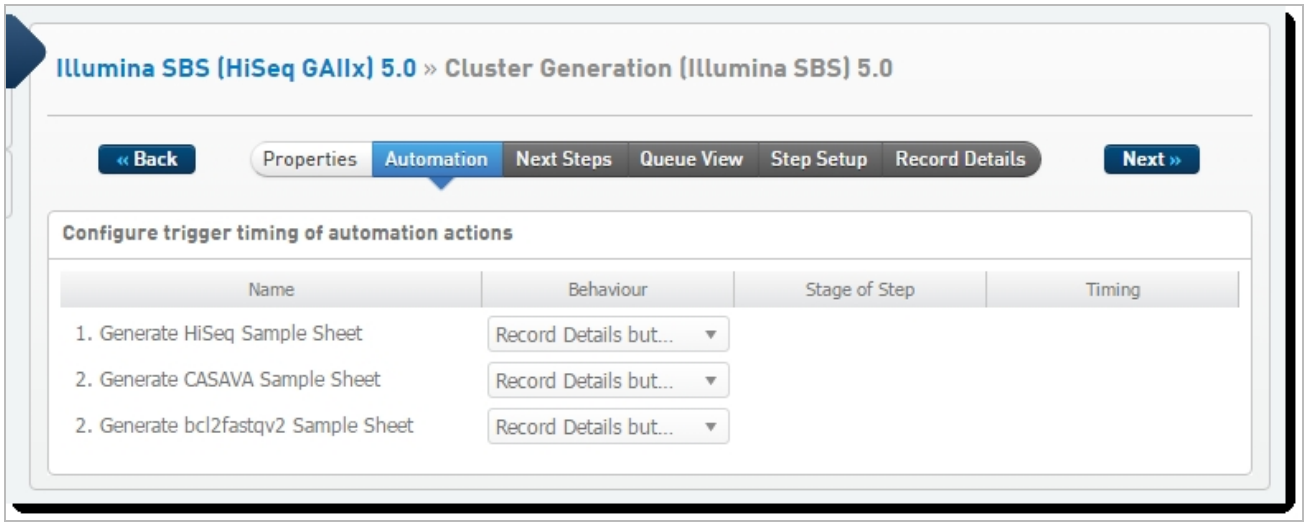
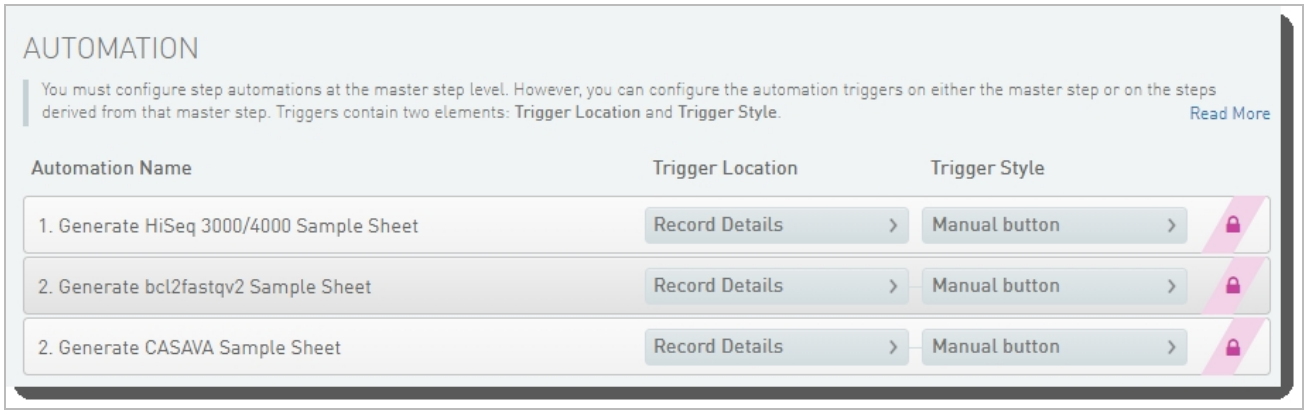


Figure 42 Figure 7: Cluster Generation I (Illumina SBS) 5.0 automation configuration - LIMS v5.x



2. Each of these automations invokes a script that generates the sample sheet – a CASAVA or bcl2fastq2 format comma-separated text file containing the required columns.
3. If the CASAVA format analysis sample sheet is used, once the sequencing run is in progress on the instrument and run data capture begins, the sample sheet for the current run is copied to the following directory for use with Illumina's Bcl to FASTQ conversion and demultiplexing tool for CASAVA:

```
<run data directory>/Data/Intensities/BaseCalls/
```

See the *CASAVA 1.8.2 Sample Sheet Generation* and bcl2fastq2 Sample Sheet Generation articles for detailed information on:

- Sample sheet generation script parameters and usage
- Sample sheet data and configuration options
- Sample sheet rules and constraints

User interaction

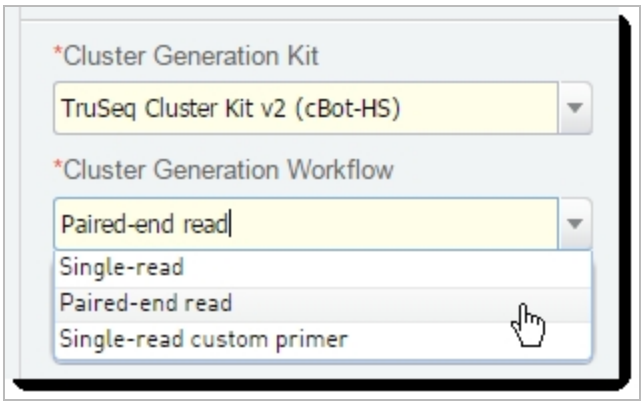
1. On the Placement screen of the Cluster Generation (Illumina SBS) 5.0 step:
    - Scan the flow cell ID (FCID) to populate the Container Name field.
    - Place the samples and proceed to Record Details screen.
2. On the Record Details screen of the Cluster Generation (Illumina SBS) 5.0 step:
    - Note that the value of the Workflow field is already set to GenerateFASTQ.

Figure 43 Figure 8: Workflow field of Cluster Generation step



    - In the Cluster Generation Kit field, select the desired kit from the drop-down list.
    - In the Cluster Generation Workflow field, select Paired-end or Single End Readfrom the drop-down list.

Figure 44 Figure 9: Cluster Generation Kit and Workflowfields in Record Details screen of Cluster Generation (Illumina SBS) 5.0 step



    - In the Reagent Lot Tracking section, select the desired TruSeq Cluster Kit from the drop-down list.
    - In the Step Details area:
3. For Paired End Reads: In the Read 2 Cycles field, enter a value other than 0.
4. Enter values for the remaining required custom fields / UDFs.
5. Click the first button – Generate HiSeq Sample Sheet – to generate the sample sheet to be uploaded to the HiSeq/GAIIx instrument.
6. Once the Generate HiSeq Sample Sheet automation has completed, the HiSeq sample sheet and log file are generated and attached to the step.

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- Download the sample sheet file and upload to the HiSeq/GAIIx sequencing control software (HCS/SCS) to start a run.

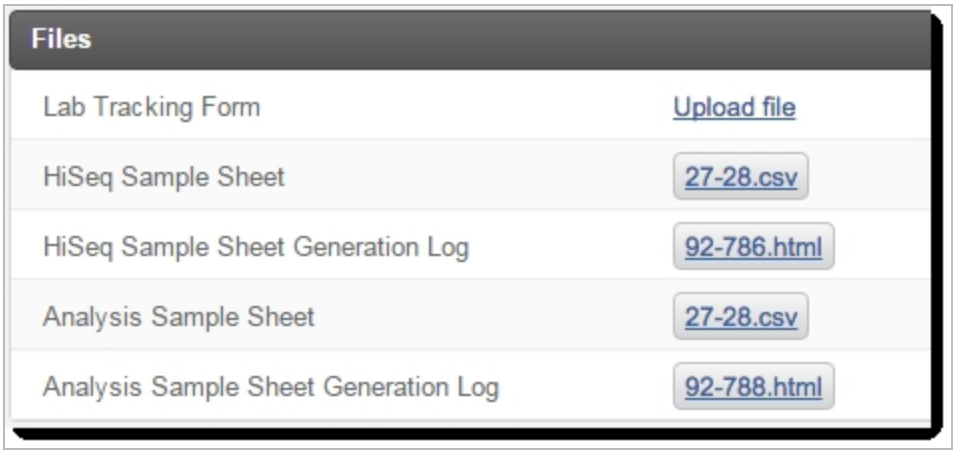
7. Click the second button to generate the analysis sample sheet. In the screenshot below, the step has been configured to generate a bcl2fastq2 format analysis sample sheet (see *Configuring Sample Sheet Generation* on page 2354).

Figure 45 Figure 10: Configured sample sheet generation automations in Record Details screen of Cluster Generation (Illumina SBS) 5.0 step



8. Once the automation has completed:
   - bcl2fastq2: Download the sample sheet file; this will be used for analysis later in the workflow.
   - CASAVA: You do not need to download this file; it will be accessed during the Bcl Conversion and Demultiplexing (Illumina SBS) 5.0 step.
   - The analysis sample sheet and log file are generated and attached to the step.
9. (Optional) Upload the Lab Tracking Form to the appropriate placeholder.

Figure 46 Figure 11: Record Details screen of Cluster Generation (Illumina SBS) 5.0 step (Files section)



Sequencing Results Parsing

The Illumina Sequencing (Illumina SBS) 5.0 step records information for each lane of the flow cell and generates a report summarizing the results. In addition, run parameters, run info, the first base report (if generated), and a link to the run folder are automatically captured.

Generated and Captured Files

Table 3 describes the run information files, reports, placeholders, and links that the LIMS automatically generates or captures during a sequencing run.

Table 3: Run information generated or captured by Illumina Sequencing (Illumina SBS) 5.0 step

Item	Description
Run Info Run Parameters (HCS-controlled instruments only) RTA Configuration XML (SCS-controlled instruments only)	These XML files are automatically captured by the LIMS from the instrument's run folder. They include the key run parameters, many of which are parsed out into key custom fields/UDFs.
Link to Run Folder	Automatically generated by the LIMS, this is a link to the network run folder where the data that was captured from the instrument during the run is stored.
Illumina Run Report	Automatically generated by the LIMS, this report provides key information about the run and the samples on the flow cell. Information includes the flow cell ID, run directory location, instrument name, as well as primary analysis metrics for the instrument – summarized per flow cell lane for the entire run, and individual reads in the case of paired-end runs. These metrics are compared against the instrument's per lane averages, calculated using metrics from the last 5 sequencing runs. Any values outside of 1 standard deviation are highlighted.
First Base Report (HCS-controlled instruments only)	Optionally generated during the instrument run by some Illumina instruments and captured by the LIMS, this report provides metrics to help determine the quality of the run. Note that manual file import is required for Sequencing Control Software (SCS)-controlled instruments.
Lab Tracking Form placeholder	This placeholder in the LIMS allows the user to manually attach a lab-specific tracking form to the step.

Metadata

Listed below is the metadata that the LIMS automatically captures from the Illumina sequencing software as part of a sequencing run (see Figure 12). This information is gathered from various run result files and events.

- Date Run* – run start date
- Instrument Run ID – sequencer instrument name
- Control Lane – PhiX control lane (blank or 1-8)
- Experiment Name – entered in software

- Finish Date* – run completion date
- Flow Cell ID
- Flow Cell Position – A or B
- Flow Cell Version
- Output Folder – run folder root
- Index 1 Read Cycles – intended Index cycles
- Index 2 Read Cycles – intended Index cycles
- Run ID – the unique run ID
- Run Type – sequencing run type description
- Status – current status of the sequencing run on the instrument
- SBS Kit Lot #
- SBS Kit Type

Date Run is set to the date that the Begin Run event file is first picked up and associated with a step in BaseSpace Clarity LIMS, not the date of the Begin Run event itself (the date the run was performed on the instrument).

Finish Date is populated as follows:

- If the End Run event contains a date in the format YYYY-MM-DD, Finish Date will be set to the date in the event file
- If the End Run event does not contain a date or the date is in the wrong format, Finish Date will be set to the date when the event file is processed

Primary analysis metrics

Table 4 lists the Real Time Analysis (RTA) primary analysis metrics the LIMS automatically captures and records, per read, for samples in each flow cell lane. Some of these metrics are captured as soon as they become available during the run; others are captured upon run completion.

Figure 47 Table 4: RTA primary analysis metrics captured by Illumina Sequencing (Illumina SBS) 5.0 step

RTA primary analysis metrics	Corresponding LIMS custom fields/UDFs
Raw Yield (Gb)	Yield PF (Gb) R1 Yield PF (Gb) R2
% Bases >Q30	% Bases >=Q30 R1 % Bases >=Q30 R2
Cluster Density (K/mm^2)	Cluster Density (K/mm^2) R1 Cluster Density (K/mm^2) R2
Clusters Raw	Clusters Raw R1 Clusters Raw R2

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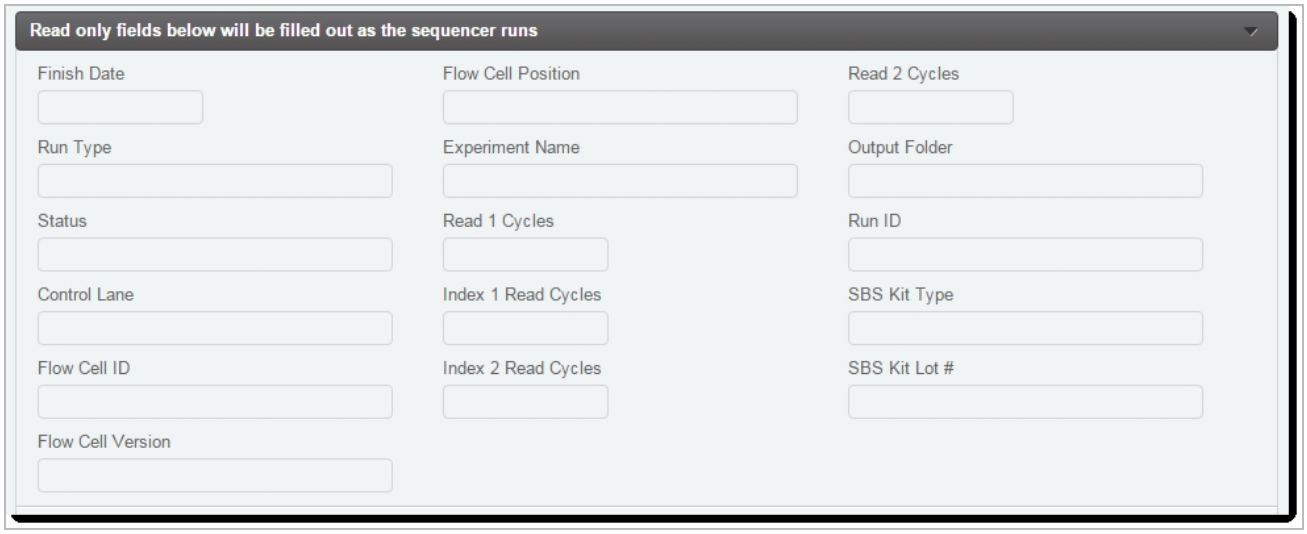
RTA primary analysis metrics	Corresponding LIMS custom fields/UDFs
Clusters PF	Clusters PF R1 Clusters PF R2
%PF	%PF R1 %PF R2
Intensity Cycle 1	Intensity Cycle 1 R1 Intensity Cycle 1 R2
% Intensity Cycle 20	% Intensity Cycle 20 R1 % Intensity Cycle 20 R2
% Phasing	% Phasing R1 % Phasing R2
% Prephasing	% Prephasing R1 % Prephasing R2
% Aligned	% Aligned R1 % Aligned R2
% Error Rate	% Error Rate R1 % Error Rate R2

How it Works

1. The sequencing service runs in the background on the LIMS server. The service detects event files that the instrument software (RTA) is producing as the run progresses; this in turn lets the service know where to find the run data.
2. As the run data is written out and the events come in (Begin Run, Cycle Complete, End Run):
    - The data is matched to the step based on the flow cell ID, which was entered by the user as the Container Name on the Cluster Generation step.
    - Read-only custom field / UDF values on the Record Details screen are populated accordingly (see Figure 12).

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Figure 48 Figure 12: Record Details screen of Illumina Sequencing (Illumina SBS) 5.0 step showing custom fields/ UDFs used for result parsing



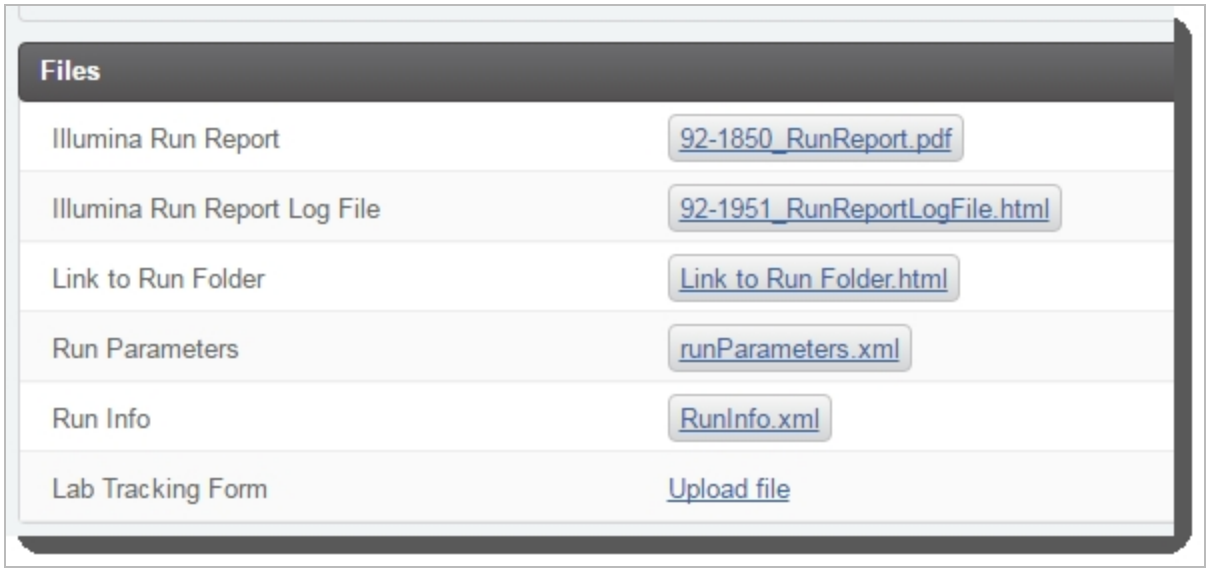
User Interaction

On the Record Details screen of the Illumina Sequencing (Illumina SBS) 5.0 step:

1. The read-only custom field/UDF values are automatically populated as the sequencer runs. Figure 12 shows the Record Details screen fields that are used for result parsing.
2. Once the run has completed:
    - The read-only custom fields/UDFs shown in Figure 12 are populated.
    - The Illumina Run Report is generated and attached.
    - The Run Parameters and Run Info files are attached.
    - The Link to Run Folder is generated and attached.
    - QC flags are set on each lane.
3. [Optional] In theFiles section, upload the First Base Report and Lab Tracking Form.

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Figure 49 Figure 13: Record Details screen of Illumina Sequencing (Illumina SBS) 5.0 step (Files section)



Bcl Conversion and Demultiplexing

How it Works

Invoking the Bcl conversion and demultiplexing automation triggers the following events:

1. Initiate Bcl conversion with the correct parameters.
2. Parse Flowcell_demux_summary.xml .
3. Attach corresponding folder link file to each LIMS sample.
4. Parse sample stats.
5. Attach Demultiplex_Stats.htm and log file.

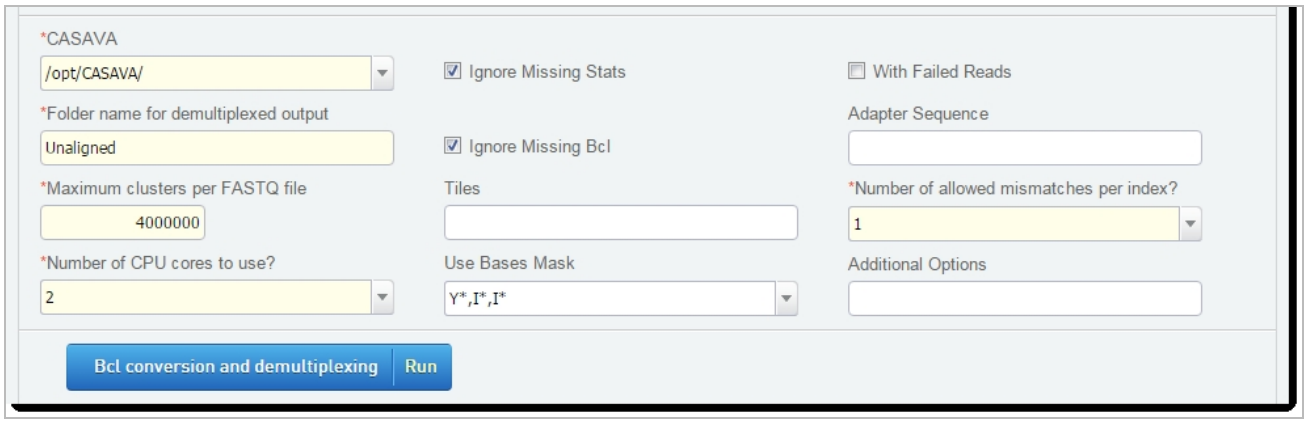
User Interaction

On the Record Details screen of the Bcl Conversion and Demultiplexing (Illumina SBS) 5.0 step:

1. Review the Bcl parameters provided, and edit as required.
2. Click Bcl conversion and demultiplexing.

Figure 14: Record Details screen of Bcl Conversion and Demultiplexing (Illumina SBS) 5.0 step

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3. Once the script has completed:
    - Demultiplex Stats and Script Log Details files are generated and attached to the step
    - HTML link files are attached to each demultiplexed sample
    - Parsed metrics are updated on the step
    - QC flags are set on the samples

Setting the InstrumentType Parameter Value

If you are upgrading to the Illumina HiSeq/GAIIx Integration Package v5.5, you will need to set the value of the instrumentType parameter (-t) in the BCL Conversion and Demultiplexing step's EPP string.

This parameter value should be set to 'hiseqga', which is the instrument type prefix of the database properties used by the string.

The correctly configured parameter will look like this:

```
-t 'hiseqga'
```

In new installations of the Illumina HiSeq/GAIIx Integration Package v5.5, this parameter value is already set in the out-of-the-box configuration.

Script Parameters and Usage

For details, see the following articles in the LIMS Integrations documentation:

- Normalization Buffer Volumes
- Assign QC flags
- CASAVA 1.8.2 Sample Sheet Generation
- Bcl2fastq2 Sample Sheet Generation
- BCL Conversion

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Components Installed with this Integration

The following sections describe the various components - files, database properties, reagent kits, and containers - that are installed by default as part of this integration. Information on installed protocols, steps, and automations is provided in the Protocols and automations section, above.

The Illumina HiSeq/GAII Integration is distributed as an RPM package that installs the components listed in Table 7. Note that this RPM package requires installation of the associated NGS Commons Package (see Release Notes).

Figure 50 Table 7: Illumina HiSeq/GAII Integration RPM package components

Component Installed	Location and Description
configure_extensions_ illumina_hiseqgaii_ workflow- v5.sh	/opt/gls/clarity/config/ Script that imports the integration configuration.
configure_extensions_ hiseqgaii_ sequencingservice- v5 .sh	/opt/gls/clarity/config/ Script that imports the database properties for the integration.
hcs-instrument-package-<*version*> ployment-bundle.zip	/opt/gls/clarity/extensions/Illumina_ HiSeq/v5/InstrumentIntegrations Instrument deployment package for HiSeq Control Software (HCS). Contains installation instructions and files to be deployed on the instrument.
scs-instrument-package-<*version*> .sh - deployment-bundle.zip	/opt/gls/clarity/extensions/Illumina_ HiSeq/v5/InstrumentIntegrations Instrument deployment package for Sequencing Control Software (SCS). Contains installation instructions and files to be deployed on the instrument.
log4j.properties, log4j.xml	/opt/gls/clarity/extensions/Illumina_ HiSeq/v5/SequencingService/conf File containing the settings for the sequencing jar's logging. Note that as of HiSeq v. 5.4, the .properties file has been replaced by the XML file.
libs directory, with multiple library jars	/opt/gls/clarity/extensions/Illumina_HiSeq/v5/SequencingService Directory containing libraries used by the sequencing jar. Obsolete as of HiSeq v. 5.4, where these libraries are packaged with the jar file.
hiseqgaii-extensions.jar	/opt/gls/clarity/extensions/Illumina_HiSeq/<version>/EPP Jar file containing API-based LIMS extensions used throughout the workflow.
hiseqgaii-sequencing.jar	/opt/gls/clarity/extensions/Illumina_HiSeq/v5/SequencingService Sequencing service jar file that captures the run results.

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Component Installed	Location and Description
hiseqgaii_seqservice-v5.sh	/opt/gls/clarity/extensions/Illumina_HiSeq/v5/SequencingService/bin Provides commands for interacting with the sequencing service. Accessible at /etc/init.d
run_hiseqgaii_seqservice	/opt/gls/clarity/extensions/Illumina_HiSeq/v5/SequencingService/bin Called by hiseqgaii_seqservice-v5, this script performs some basic system validation, then starts the sequencing service.

Database Properties Installed

Table 8 lists the database properties installed with the Illumina HiSeq/GAIIx Integration Package.

Note that several additional properties, each with the '99' suffix appended to their name, are also installed. These are intended for use by the BaseSpace Clarity LIMS Support team in automated validation tests and are not listed in the table.

Constraints

Sequencing runs are matched using the flow cell ID and the sequencing step's base name – Illumina Sequencing (Illumina SBS).

Do not change this base name – it is expected by the sequencing service that captures instrument run results. The base name is stored in the sequenceProcessBaseName database property (see Table 8). If this name is changed without the database property being updated, the 'flow cell ID <-> sequencing step base name' matching system will fail.

If required, you may modify the step name by editing or adding to the text *after* the base name portion as this is not used in the matching system. For example, you could change Illumina Sequencing (Illumina SBS) 5.0 to Illumina Sequencing (Illumina SBS) v5.

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Figure 51 Table 8: Database properties installed with the Illumina HiSeq/GAIIx Integration Package

Property	Description	Default Value
hiseqga.seqservice.ignoreUnmatchedContainerIds	A flag indicating if event files that cannot be matched to flow cells in the LIMS should be archived after a certain period of time (true), or continually reprocessed (false).	false To prevent the gls_events file directory from becoming cluttered, it is recommended that the value of this property is set to 'true'.
hiseqga.seqservice.ignoreUnmatchedContainerIdsWaitDays	The number of days between when the event is created and the event file is archived.	14
hiseqga.seqservice.synchronizationPeriod	Invocation period in seconds.	60
hiseqga.seqservice.sequenceProcessBaseName	Sequencing process type base display name. Partial matching is used to look up the process type.	Illumina Sequencing (Illumina SBS)
hiseqga.seqservice.eventFileDirectory.1	A network location monitored for event files.	/mnt/gls_events
hiseqga.seqservice.netPathPrefixSearch.1	The network directory prefix contained in the event file.	\\nas\network\run_data
hiseqga.seqservice.netPathPrefixReplace.1	The mapped network directory mount name on the server used to access the run data directory.	/mnt/run_data

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Property	Description	Default Value
hiseqga.seqservice.eventFileDirectorySuffixes	A list of eventFileDirectory path entries to monitor for event files. The value is one or more comma separated integers.	99
hiseqga.seqservice.runReportViewsVersion	The current version of the Run Report views in the database. The value 0 represents the state before the views are created. This property is automatically updated by the run report.	0
hiseqga.seqservice.netPathPrefixSearchReplaceSuffixes	A list of netPathPrefix search and replace entries for transforming Windows to Linux network paths. The value is one or more comma-separated integers.	99
hiseqga.bcl.netPathPrefixSearch.1	(BCL conversion) The network directory prefix contained in the event file.	\\nas\network\run_data
hiseqga.bcl.netPathPrefixReplace.1	(BCL conversion) The mapped network directory mount name on the server used to access the run data directory.	/mnt/run_data
hiseqga.bcl.replacementSuffixes.bclserver	(BCL conversion) A list of network path replacements associated with the BCL EPP Channel. The value is one or more comma-separated integers. The property format is hiseqga.bcl.replacementSuffixes.<EPP_CHANNEL_NAME>	99

Support for multiple seqservice.netPathPrefixSearch property values

It is possible to configure support for multiple, identical seqservice.netPathPrefixSearch property values. For details, see Configuring Multiple Identical netPathPrefixSearch values.

Reagent Kits Installed

The following reagent kits are included in the default configuration for the Illumina HiSeq/GAIIx Integration:

- AMPure XP Beads

- TruSeq Cluster Kit
- TruSeq Exome Enrichment - Box 1
- TruSeq Exome Enrichment - Box 2

Control Types Installed

The following control types are included in the default configuration for the Illumina HiSeq/GAIIx Integration:

- Endogenous Positive Control
- Exogenous Positive Control
- No Amplification Control
- No Reverse Transcriptase Control
- No Template Control
- PhiX v3

Container Types Installed

The following container types are included in the default configuration for the Illumina HiSeq/GAIIx Integration:

- Illumina Flow Cell
- Illumina Rapid Flow Cell

Instrument Integration

To ensure that your Illumina instrument warranty remains valid, the instrument integration must be performed and maintained by the BaseSpace Clarity LIMS Support team. To perform this integration, the Support team will require direct access to the instrument via WebEx or Remote Desktop while the instrument is idle.

Below is an overview of the steps performed by the LIMS support team when configuring the instrument for use with the Illumina HiSeq/GAIIx Integration v5.5 to BaseSpace Clarity LIMS.

Configuring the Illumina Instrument

1. Create directory on the local computer to hold the batch files. These batch files write event files to the network attached storage (NAS) share(s).
2. Create directory on the NAS to hold the event files.
3. Modify Illumina software configuration files to call the batch files that create the event files.
4. Update sequencing service default database properties to match the specifics of the installation.

Configuring Instruments

We recommend configuring sequencer instrument names in the LIMS to track the specific lab instrument on which a run was completed.

This information should be c onfigured on the Illumina Sequencing(Illumina SBS)5.0 step, and should be set to the same name as is configured in the Illumina sequencing software – i.e., this will be value of the Instrument Run ID field described in the *Metadata* on page 2343 section above.

When the integration reads the Instrument Run ID field in the run results, it will update the instrument on the step in the LIMS, eliminating the need for the user to manually enter this information.

For instructions on configuring instrument names, refer to the following articles:

LIMS v4.1 and v4.2 Documentation:

- *Adding Instruments and Instrument Types* (System Configuration section)

LIMS v5 Documentation:

- *Adding and Configuring Instruments* (Consumables section)

Configuring Sample Sheet Generation

Sample sheet generation occurs on the step prior to the sequencing run – Cluster Generation (Illumina SBS) 5.0 – which is the step where samples are placed on the container that will be loaded in the instrument.

The out-of-the-box configuration provides three automations (listed below in Table 3). These generate the following:

- One sample sheet for use with the HiSeq/GAII(x) instrument sequencing control software (CASAVA v1.8.2 format).
- Two options for an analysis sample sheet for use with CASAVA v1.8.2 or bcl2fastq v2.15 downstream analysis – at least one of which should be disabled when the system is initially configured (instructions provided below). If you do not require a sample sheet for use in downstream analysis, you may prefer to disable both options.

By default, one instrument and one analysis sample sheet are created for the flow cell loaded during the Cluster Generation (Illumina SBS) 5.0 step.

If required, you may configure the step to generate analysis sample sheets for multiple flow cells. For details, refer to the sample sheet generation article for your analysis software:

- *CASAVA 1.8.2 Sample Sheet Generation*
- *Bcl2fastq2 Sample Sheet Generation (NGS v5.16 and earlier)*

The sample sheet generation automations are configured on the Record Details screen of the Cluster Generation (Illumina SBS) 5.0 step.

Figure 52 Figure 15: Record Details screen of Cluster Generation step showing out-of-the-box sample sheet generation automations prior to configuration

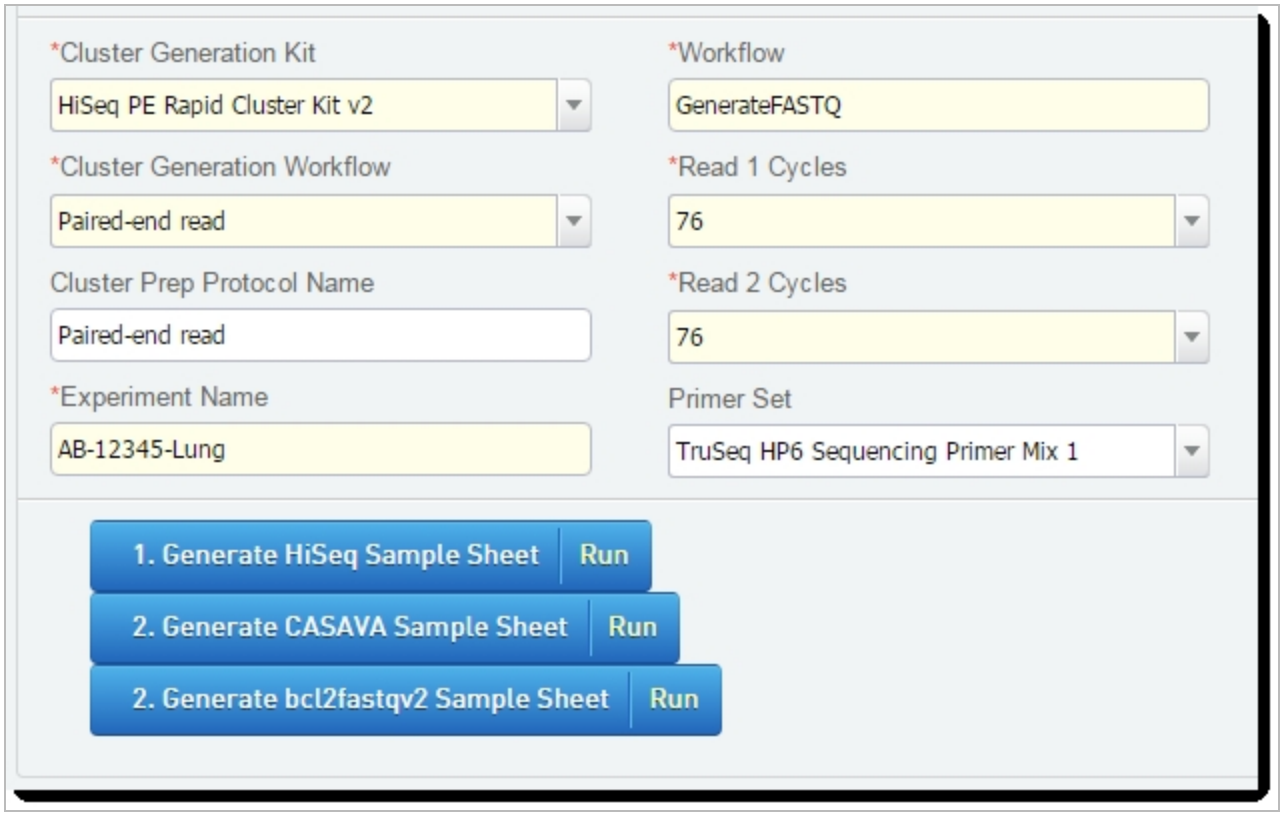


Table 5 shows the format of the sample sheets generated by each of these automations.

Figure 53 Table 5: Sample sheet generation automations and file formats

Automation	Generated Sample Sheet Format
1. Generate HiSeq Sample Sheet	CASAVA v1.8.2
2. Generate CASAVA Sample Sheet	CASAVA v1.8.2
2. Generate bcl2fastqv2 Sample Sheet	bcl2fastq v2.15

Upgrading Cluster Generation (Illumina SBS) 5.0 Master Step Custom Fields/Step UDFs

If you are upgrading to the Illumina HiSeq/GAIIx Integration Package v5.5, note the following:

- To enable generation of the bcl2fastq2 sample sheet, additional fields / UDFs have been added to the Cluster Generation (Illumina SBS) 5.0 step. These are listed in Table 6.

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Figure 54 Table 6: Cluster Generation (Illumina SBS) 5.0 - additional master step fields/step UDFs

Name	Type	Field Constraints	Preset Values
Adapter	Single-line Text	Required	
Adapter Read 2	Single-line Text		
Read 1 Cycles	Numeric	Required, minimum value 0	151, 76, and 51
Read 2 Cycles	Numeric	Required, minimum value 0	151 and 76
Experiment Name	Single-line Text	Required	
Workflow	Single-line Text	Required Use first Preset Value as the default value option selected	GenerateFASTQ

- The TruSeq Cluster Generation Kit master step field/step UDF has been renamed Cluster Generation Kit and its preset values have been updated as follows:
  - TruSeq Cluster Kit v2 (cBot-HS)
  - TruSeq Cluster Kit v3 (cBot-HS)
  - HiSeq PE Cluster Kit v4
  - HiSeq SR Rapid Cluster Kit v2
  - HiSeq PE Rapid Cluster Kit v2
- The master step fields / step UDFs Cluster Generation Kit and Cluster Generation Workflow are now required.

Disable bcl2fastqv2 and Use the CASAVA Sample Sheet for Analysis

LIMS 4.x

1. In the LIMS Web Interface, on the Configuration screen, click Protocols.
2. Edit the Illumina SBS (HiSeq GAIIx) 5.0 protocol.
3. Edit the Cluster Generation (Illumina SBS) 5.0 step.
4. On the Automation tab, set the value for 2. Generate bcl2fastqv2 Sample Sheet to "Not used."

For Research Use Only. Not for use in diagnostic procedures.

Figure 55 Figure 16: Disabling generation of the bcl2fastqv2 sample sheet

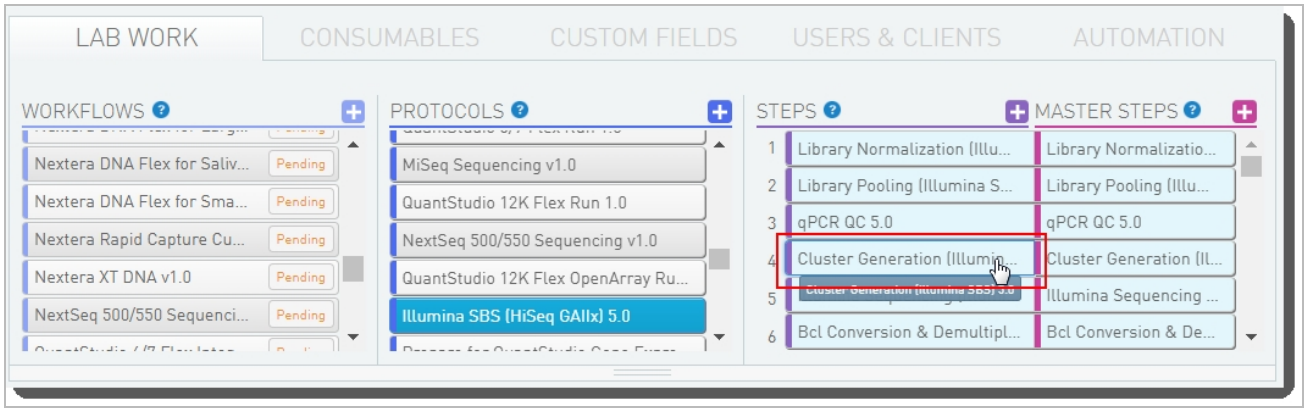


5. Save the step configuration.

LIMS 5.x

1. In the LIMS, click the Configuration menu item and then click the Lab Work tab.
2. On the Lab Work configuration screen, in the Workflows, Protocols, Steps, and Master Steps navigation panel, select (click) the Illumina SBS (HiSeq GAIIx) 5.0 protocol. The Steps and Master Steps lists update, highlighting the steps included in the protocol, and the master steps on which those steps are based.
3. Select the Cluster Generation (Illumina SBS) 5.0 step.

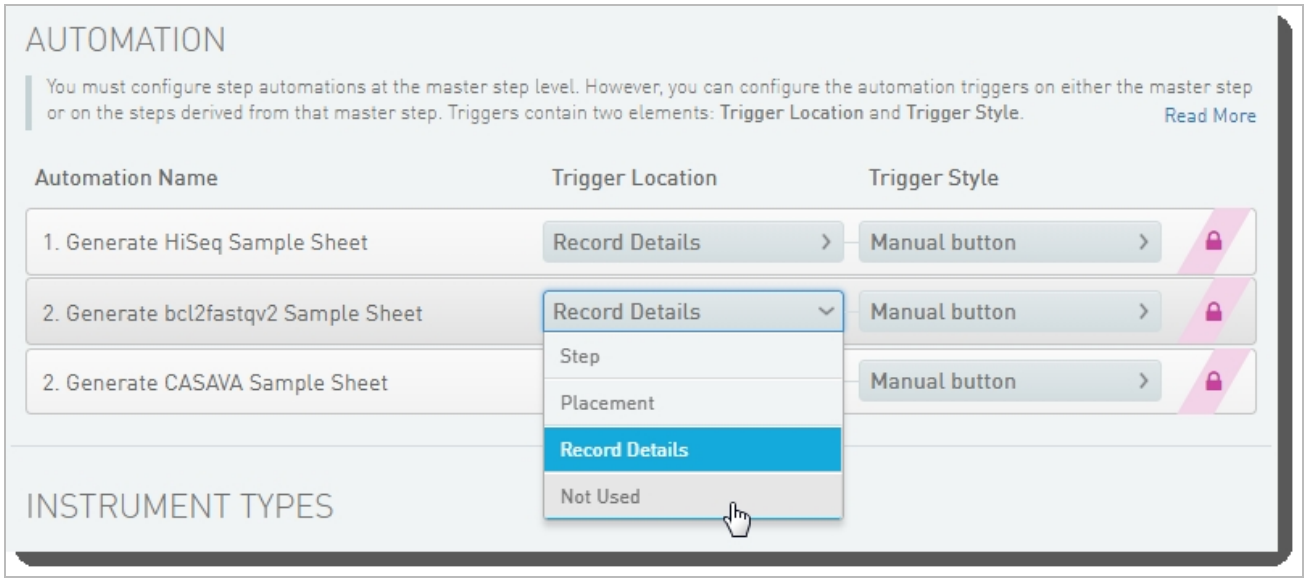
Figure 56 Figure 17: Selecting the Cluster Generation (Illumina SBS) 5.0 step



4. Below the navigation panel, the step and master step configuration forms display. Scroll down to the Automation configuration section. For the 2. Generate bcl2fastqv2 Sample Sheet automation, set the Trigger Location to Not Used.

For Research Use Only. Not for use in diagnostic procedures.

Figure 57 Figure 18: Disabling generation of the bcl2fastqv2 sample sheet

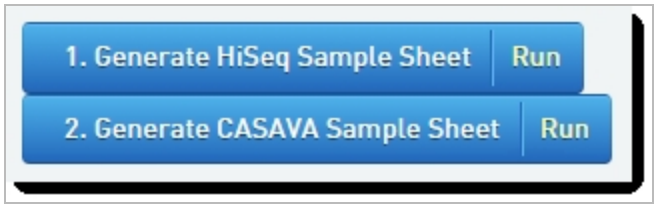


5. Save the step configuration.

After completing the configuration steps above, when the step is run, only two buttons will be visible in Record Details view.

Note that both generate the same CASAVA format sample sheet.

Figure 58 Figure 19: Automations configured for use with HiSeq/GAII and CASAVA downstream analysis

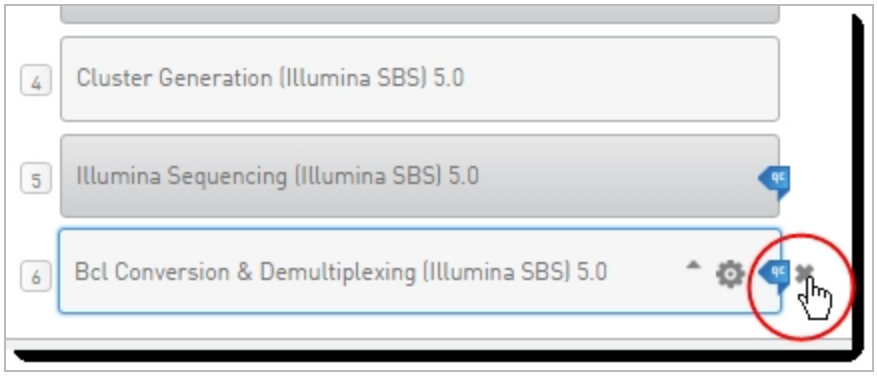


Disable CASAVA and Use the bcl2fastq2 Sample Sheet for Analysis

LIMS 4.x

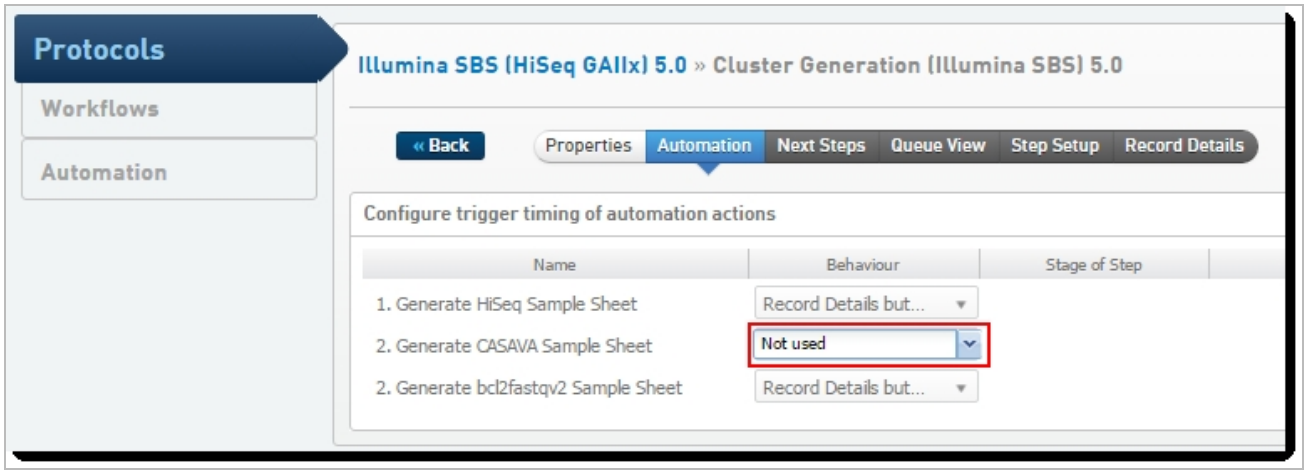
1. In the LIMS Web Interface, on the Configuration screen, click Protocols .
2. Edit the Illumina SBS (HiSeq GAIIx) 5.0 protocol.
3. Remove the BCL Conversion and Demultiplexing (Illumina SBS) 5.0 step.

Figure 59 Figure 20: Removing BCL Conversion and Demultiplexing from Illumina SBS (HiSeq GAIIx) protocol



4. Save the protocol configuration.
5. Edit the Cluster Generation (Illumina SBS) 5.0 step.
6. On the Automation tab, set the value for 2. Generate CASAVA Sample Sheet to "Not used."

Figure 60 Figure 21: Disabling generation of the CASAVA sample sheet

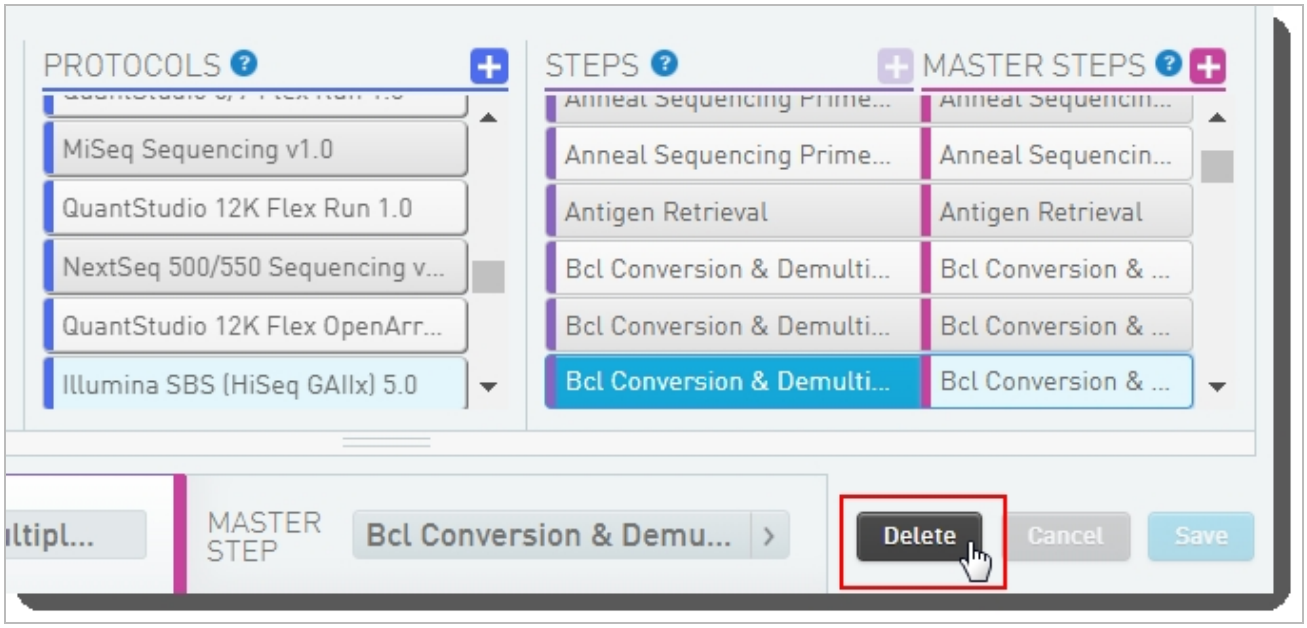


7. Save the step configuration.

LIMS 5.x

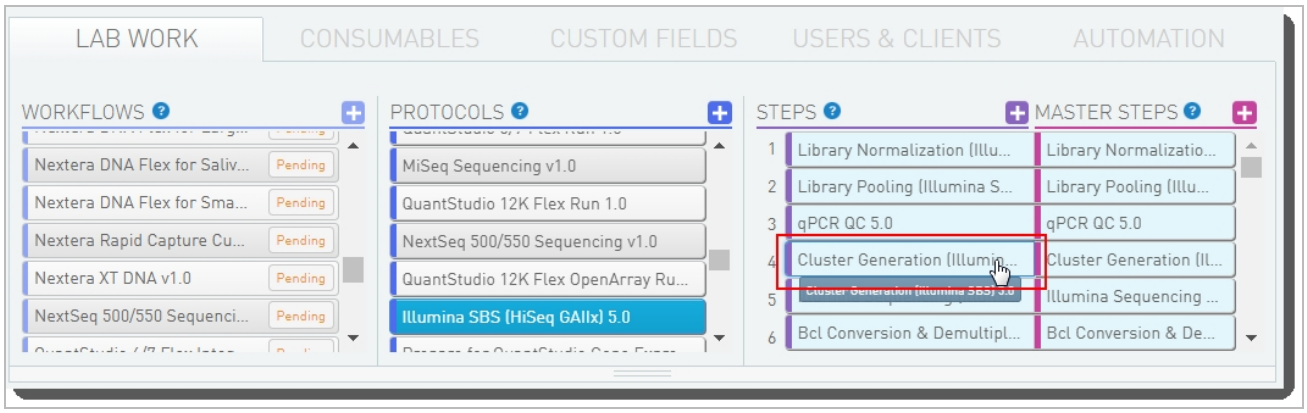
1. In the LIMS, click the Configuration menu item and then click the Lab Work tab.
2. On the Lab Work configuration screen, in the Workflows, Protocols, Steps, and Master Steps navigation panel, select (click) the Illumina SBS (HiSeq GAIIx) 5.0 protocol. The Steps and Master Steps lists update, highlighting the steps included in the protocol, and the master steps on which those steps are based.
3. Select the BCL Conversion and Demultiplexing (*Illumina SBS) 5.0* step and click Delete.

Figure 61 Figure 22: Removing BCL Conversion and Demultiplexing from Illumina SBS (HiSeq GAIIx) 5.0 protocol



4. Save the protocol configuration.
5. In the Steps list, select the Cluster Generation (Illumina SBS) 5.0 step.

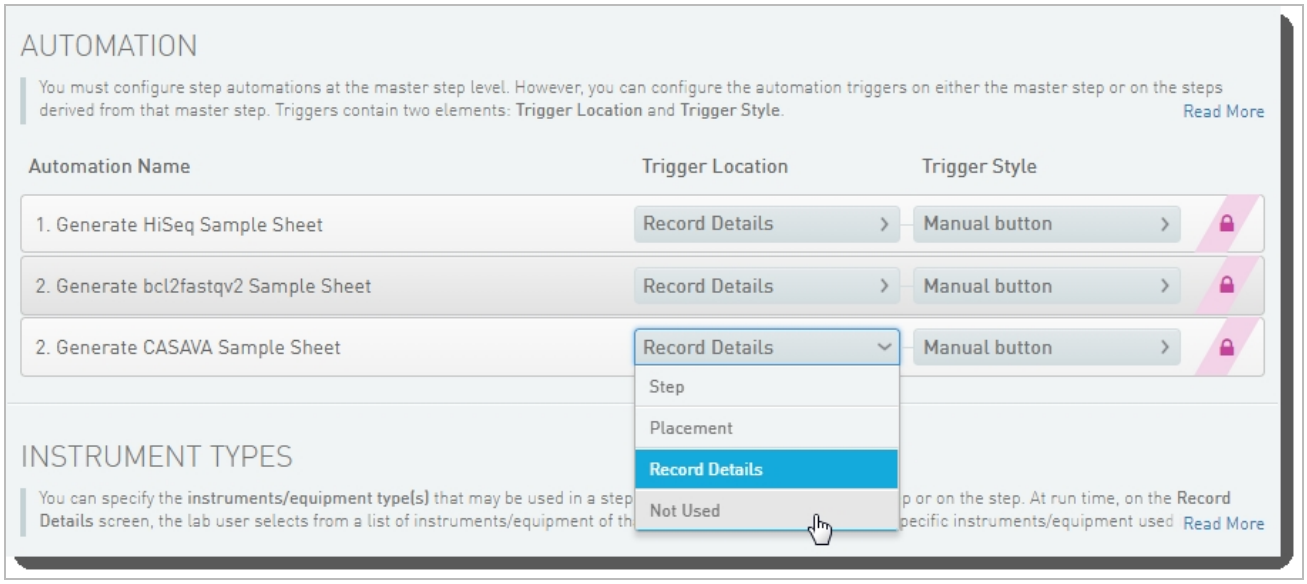
Figure 62 Figure 23: Selecting the Cluster Generation (Illumina SBS) 5.0 Step



6. Below the navigation panel, the step and master step configuration forms display. Scroll down to the Automation configuration section. For the 2. Generate CASAVA Sample Sheet automation, set the Trigger Location to Not Used.

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Figure 63 Figure 24: Disabling Generation of the CASAVA Sample Sheet



7. Save the step configuration.

After completing the configuration steps above, when the step is run, only two buttons will be visible in Record Details view.

Figure 64 Figure 25: Automations configured for use with HiSeq/GAIIx and bcl2fastq2 downstream analysis



See the *CASAVA 1.8.2 Sample Sheet Generation* and *Bcl2fastq2 Sample Sheet Generation (NGS v5.16 and earlier)* articles for detailed information on:

- Sample sheet generation script parameters and usage
- Sample sheet data and configuration options
- Enabling sample sheet generation for multiple flow cells
- Enabling unique FASTQ file names per sequencing run
- Sample sheet rules and constraints

Support for container types

All one-dimensional container types with both numeric rows and numeric columns are supported.

Rules and Constraints

This integration operates with the following constraints:

- The flow cell ID must be unique. There should not be multiple flow cell containers in the system with the same name.
- The flow cell ID must be scanned as the flow cell Container Name on the Cluster Generation (Illumina SBS) 5.0 step.
- The sample sheet provided in the BaseCalls directory must be named SampleSheet.csv.
- For sample sheet generation constraints, refer to the appropriate article for your analysis software:
  - *CASAVA 1.8.2 Sample Sheet Generation*
  - *Bcl2fastq2 Sample Sheet Generation (NGS v5.16 and earlier)*

Vendor Resources

- Illumina HiSeq 2500 System information
- Guided sample sheet creation and setup
- Illumina Experiment Manager User Guide
- Illumina Experiment Manager Support

Illumina HiSeq/GAIIx Integration v5.5 / v5.6 Validation and Troubleshooting

Compatibility: See *Illumina HiSeq/GAIIx Release Notes (for Clarity LIMS 4.0 or later)* on page 2332

After installing and configuring the Illumina HiSeq/GAIIx Integration Package v5.5, it is recommended that you run two validation tests:

1. To confirm that automated sample sheet generation functioning correctly
2. To confirm that event files are created by the batch files in the destination path

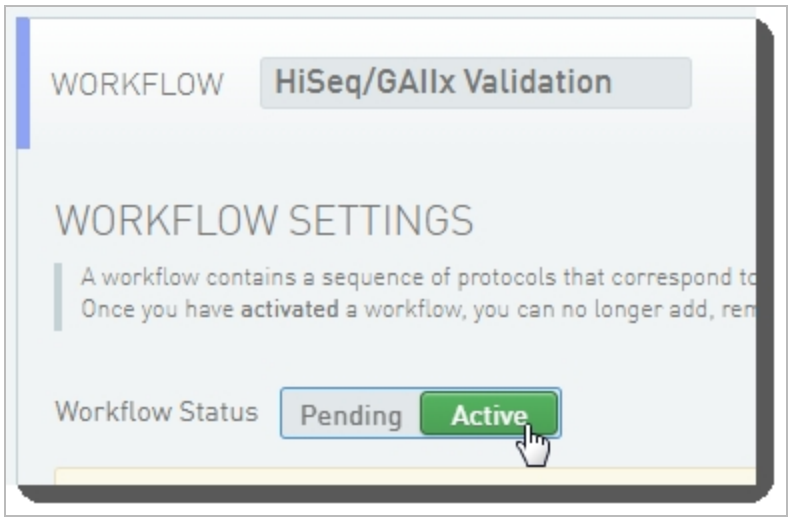
Instructions for configuring sample sheet generation to meet the needs of your lab are provided in the Illumina HiSeq/GAIIx Integration v5.5 User and Configuration Guide.

Validating Sample File Generation

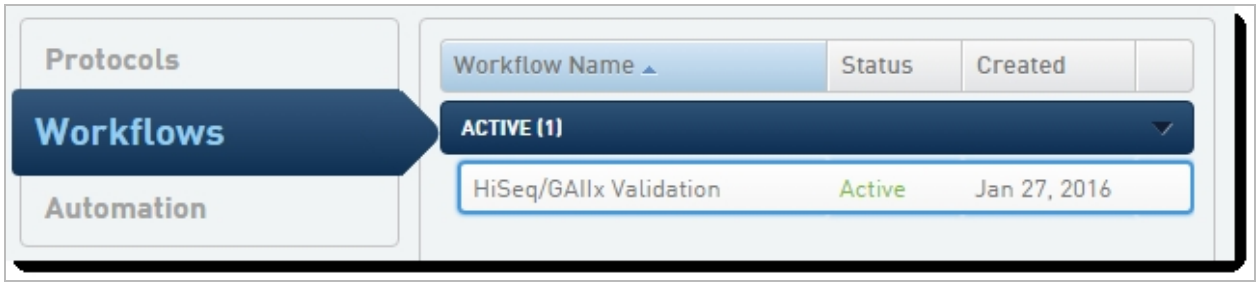
The HiSeq/GAIIx Validation workflow allows for simple validation of the sample sheet generation automations.

The following steps assume that the default configuration has already been successfully imported.

1. Activate the workflow:In the LIMS configuration area, activate the HiSeq/GAIIx Validation workflow.

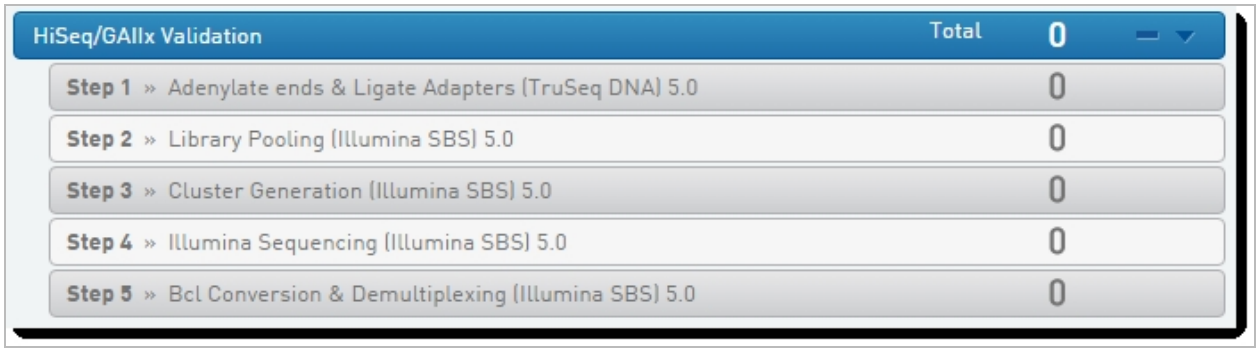


Workflow configuration and activation - LIMS v5.x

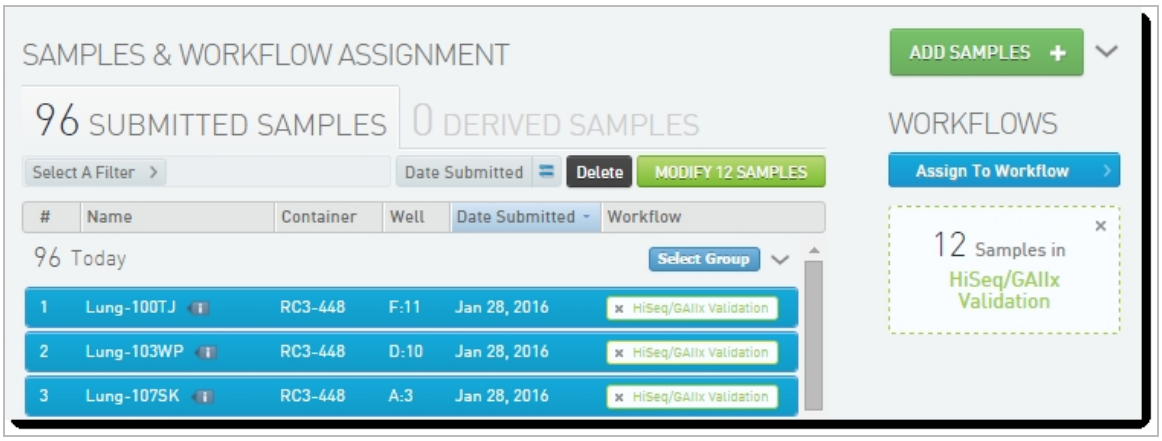


Workflow configuration and activation - LIMS v4.x

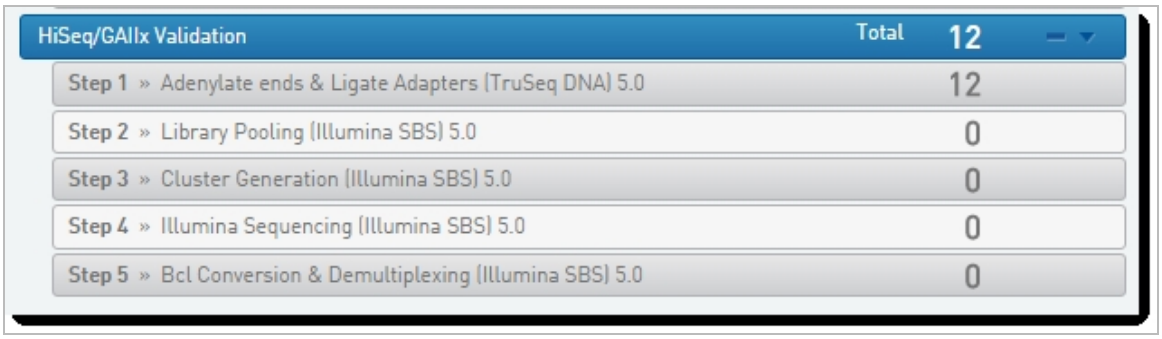
The HiSeq/GAIIx Validation protocol will now display in Lab View.



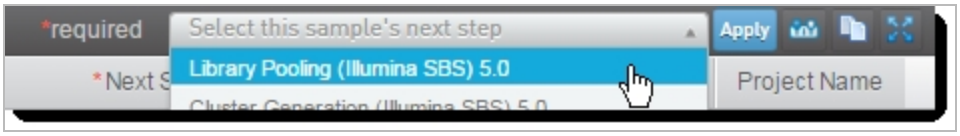
2. On the Projects and Samples screen:
    - Create a test project and add samples to it.
    - Assign your samples to the HiSeq/GAIIx Validation workflow.



3. In Lab View, locate the HiSeq/GAIIx Validation protocol:
    - You should see your samples queued for the Adenylate ends and Ligate Adapters (TruSeq DNA) 5.0 step.



    - Add the samples to the Ice Bucket and begin work.
    - Run through the steps until you reach the Assign Next Steps screen. Assign the samples to the Library Pooling (Illumina SBS) 5.0 step.



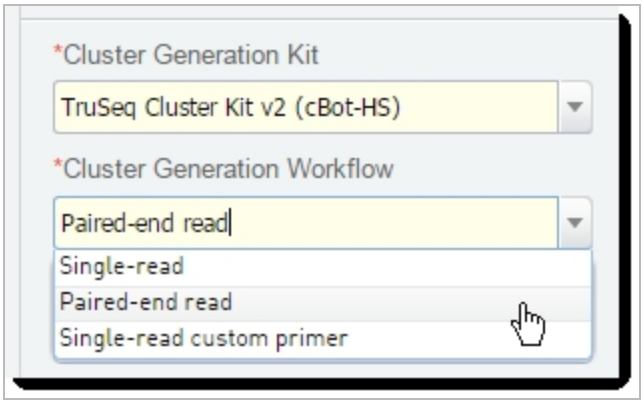
4. Return to Lab View and locate the HiSeq/GAIIx Validation protocol:
    - You should see your samples queued for the Library Pooling (Illumina SBS) 5.0 step.
    - Add the samples to the Ice Bucket and begin work.
    - Run through the steps until you reach the Assign Next Steps screen. Assign the samples to the Cluster Generation (Illumina SBS) 5.0 step.
5. Return to Lab View and locate the HiSeq/GAIIx Validation protocol:
    - You should see your samples queued for the Cluster Generation (Illumina SBS) 5.0 step.
    - Add the samples to the Ice Bucket and begin work.
    - Run through the steps until you reach the Record Details screen.

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6. On the Record Details screen of the Cluster Generation (Illumina SBS) 5.0 step:
    - Note that the value of the Workflow field is already set to GenerateFASTQ.



    - In the Cluster Generation Kit field, select the desired kit from the drop-down list.
    - In the Cluster Generation Workflow field, select the desired workflow from the drop-down list.



    - In the Reagent Lot Tracking section, select the desired TruSeq Cluster Kit from the drop-down list.
    - In the Step Details area:
    - For Paired-end reads: In the Read 2 Cycles field, enter a value other than 0.
    - Enter values for the remaining required custom fields.
7. Click the sample sheet generation buttons to invoke the automations configured on this step and generate the sample sheets.
   In the screenshot below, the step has been configured to generate the bcl2fastq2 analysis sample sheet. See Configuring sample sheet generation section of the *Illumina HiSeq/GAIIx Integration v5.5 / v5.6 Configuration and User Interaction* on page 2333.



8. The scripts should complete successfully and attach the appropriate sample sheets and log files.
9. Download the sample sheet files and log files and validate their format and content:
    - *CASAVA 1.8.2 Sample Sheet Generation*

- *Bcl2fastq2 Sample Sheet Generation (NGS v5.16 and earlier)*
- Access the Sample Sheet Generation article for your analysis software and review the File format and contents section:
- Bcl2fastq2-format sample sheet only: If dual indexes were added to the samples during the Adenylate ends and Ligate Adapters (TruSeq DNA) 5.0 step, confirm that the index2 column is present and correctly populated.

10. Complete the step.
11. Complete the remaining steps in the protocol and mark the protocol as complete.

Validating That Event Files are Created by the Batch Files in the Destination Path

This test validates that:

1. Your DESTINATION_PATH is correctly configured.
2. The instrument computer can access and write to the DESTINATION_PATH.
3. There are no syntax errors in the LIMS batch files.

The steps below assume that the default configuration has already been successfully imported.

The HiSeq/GAII sequencing service running on the LIMS server consumes (i.e. processes and then archives) the event files, making it difficult to perform validation while it is running.

- If the sequencing service is running on the LIMS server, you can temporarily modify the FINAL_EXTENSION value in the LIMS batch files from .txt to .test. The sequencing service will only consume .txt files and will leave all other extensions alone. Don't forget to change the FINAL_EXTENSION back to .txt after your manual validation.
- Alternatively, if you have access to the LIMS server, you can monitor the HiSeqGAIntegrator.log, which logs the file name and contents of each event file it processes.
  You may use the following command to do this: /etc/init.d/hiseqgaii_seqservice-v5 log

Steps

1. On the instrument, in C:\gls, double-click gls_event_hcs.bat to execute the file.
2. Confirm that an 'empty' event file appears in the configured DESTINATION_PATH.
3. In C:\gls, double-click gls_event_hcs_rta.bat.
4. Confirm that an 'empty' event file appears in the configured DESTINATION_PATH.

Manual invocation of gls_event_hcs.bat will produce output resembling:

```
Filename:
event--07295964.test
Contents:
```


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```
cycleNumber =
runFolder =
netFolder =
runType =
eventType =
softwareType = HCS
```

Manual invocation of gls_event_hcs_rta.bat will produce output resembling:

```
Filename:
event-EndRun-07300169.test
Contents:
cycleNumber =
runFolder =
netFolder =
readType =
eventType = EndRun
softwareType = HCS
finishDate = 2016-02-16
```

Troubleshooting

If an automation trigger does not appear to run its corresponding scripts, see:

- *Troubleshooting Automated Informatics* in the LIMS documentation (Automated Informatics section)
- Troubleshooting EPP/Automation in the API > Overview > API Advanced Topics section

If an error occurs that does not provide direction on how to proceed:

1. Confirm the version of the installed Illumina HiSeq/GAII Integration Package by running the following command from the server console:

```
java -jar /opt/gls/clarity/extensions/Illumina_HiSeq/v5/EPP/hiseqgaii-
extensions.jar
```

2. If the error is related to retrieving run results data, review the HiSeqGAIntegrator.log file.
3. Contact the BaseSpace Clarity LIMS Support team, supplying the relevant information from the troubleshooting already performed.

Additional troubleshooting information for this integration is provided on the *Illumina Instrument Integrations FAQ* on page 2598 page.

Illumina HiSeq/GAIIx Instrument Interface

To ensure that your Illumina instrument warranty remains valid, this integration must be performed and maintained by the Platform SW Clarity LIMS support team as needed.

Overview of the Platform SW Clarity LIMS ↔ Illumina HiSeq/GAIIx Instrument Interface

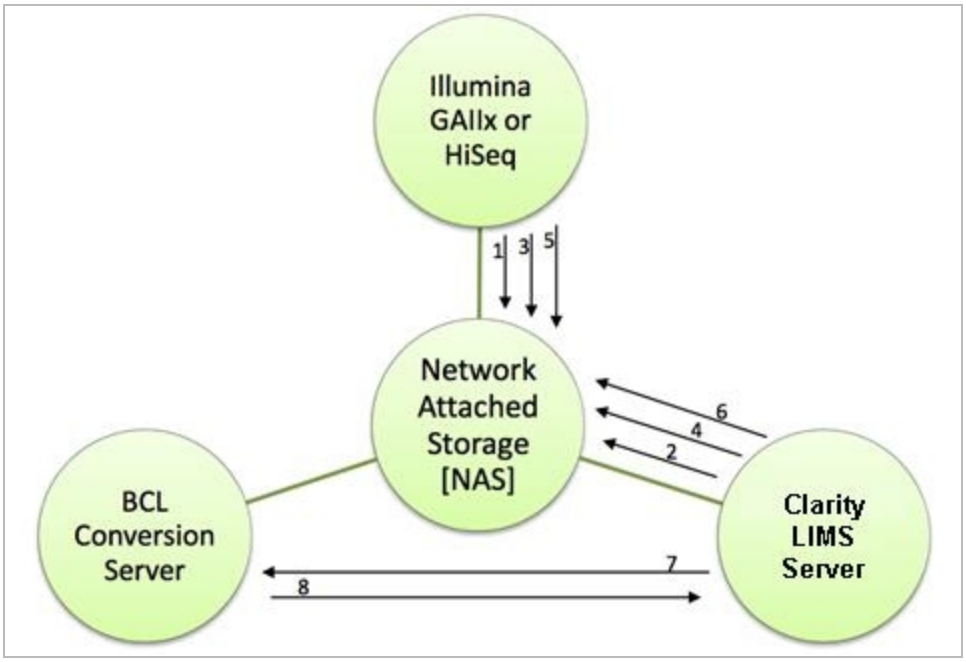


Image Action #	Description
0	Illumina Sequencing (Illumina SBS) 4.0 initiated in Platform SW Clarity LIMS.
1	1. Begin Run event fires on Illumina instrument. 2. Instrument fires configured LIMS script. 3. Script writes Begin Run event to Network Attached Storage (NAS) root folder in `\gls_events` .

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Image Action #	Description
2	1. Timer-based daemon on Clarity LIMS server wakes up and looks for events. 2. Daemon discovers Begin Run event and processes it. 3. Daemon uses the supplied process run directory to locate the run folder on the NAS. 4. Daemon parses key information from the run folder, including the Flowcell ID. 5. Daemon uses Clarity LIMS REST API to do the following: – Locate the sequencing process for this flow cell (based on ID). – Populate process UDFs: • Run Type, Control Lane, Flowcell ID, Flowcell Position, Flowcell Version, Experiment Name, Read 1 Cycles, Index 1 Read Cycles, Index 2 Read Cycles, Read 2 Cycles, Output Folder, Run ID, TruSeq SBS Kit lot #, TruSeq SBS Kit Type – Examine list of files to collect, and attach any that are now available: • `RunInfo.xml`, `runParameters.xml` (or `RTAConfiguration.xml`), Link to Run Folder.html
3	1. Cycle Complete event fires on Illumina instrument. 2. Instrument fires configured Clarity LIMS script. 3. Script writes Cycle Complete event to NAS network root folder in `\gls_events` .

Image Action #	Description
4	1. Timer-based daemon on Clarity LIMS server wakes up and looks for events. 2. Daemon discovers Cycle Complete event and processes it. 3. Daemon uses the supplied process run directory to locate the run folder on the NAS. 4. Daemon parses key information from the run folder, including the Flowcell ID. 5. Daemon uses Clarity LIMS REST API to do the following: – Populate process UDFs: • Status – populate with cycle information (e.g., Cycle 18 of 36) – Examine list of files to collect, and attach any that are now available: • `First_Base_Report.htm`. • Re-checks for any other files that may not have been available at Begin Run event. – On Cycle 25 of standard output runs, Clusters PF R1 and %PF R1 run statistics are calculated from the binary InterOp files and stored in Output UDFs on the sample analytes in the flow cell (generated by Cluster Generation).
5	1. End Run event fires on Illumina instrument. 2. Instrument fires configured Clarity LIMS script. 3. Script writes End Run event to NAS network root folder in `\gls_events`.

For Research Use Only. Not for use in diagnostic procedures.

Image Action #	Description
6	1. Timer-based daemon on Clarity LIMS server wakes up and looks for events. 2. Daemon discovers End Run event and processes it. 3. Daemon uses the supplied process run directory to locate the run folder on the NAS. 4. Daemon parses key information from the run folder including the Flowcell ID . 5. Daemon uses Clarity LIMS REST API to do the following: – Populate process UDFs: • Finish Date • Status – Examine list of files to collect, and attach any that are now available: • Final check for any files available that are not already attached. – Run statistics are parsed from several binary InterOp files and are stored (visible in the Illumina Sequencing run). – Run Report invoked, generated, and attached. T his report highlights metrics with values that are >1 std. deviation outside the average calculated from the past 5 runs on that specific instrument.
7	After sequencing, a lab scientist runs a Bcl conversion process invoking EPP to: 1. Retrieve necessary system data via the REST API and the network run directory from the sequencing process. 2. Invoke Bcl conversion using previously stored network run directory, `SampleSheet.csv` (generated by theCluster Generation process) file, and any parameters specified in process UDFs. 3. Demultiplexing of data takes place directly within the network run directory by the Illumina Bcl software—as would be the case if Clarity LIMS did not initiate it.

For Research Use Only. Not for use in diagnostic procedures.

Image Action #	Description
8	At the conclusion of the EPP dispatched job (and the Bcl conversion it initiates) some lightweight but important data is captured: • HTML Link files to the FASTQ reads (the actual FASTQ reads are now in the run directory on the NAS) • `Demultiplex_Status.csv` • Script Log Details

Manually Creating HCS Event Files

This section explains how to manually create the HiSeq Control Software (HCS) event files.

BeginRunEvent

Source Data

runParameters.xml

The following variables are found in the runParameters.xml file:

- <TempFolder>
- <OutputFolder>
- <RunID>

RunInfo.xml

- [runType] - from <reads> information gleaned from RunInfo.xml.]

This value must be one of the following:

- If there are three <Read Number> lines:

```
Paired End Indexing Run
```

- If there is one <Read Number> line:

```
Single Read Run
```

Pattern

```
gls_event_hcs.bat 0 "<TempFolder>" "<OutputFolder>\<RunID>" "[runType]"
BeginRunHCS
```

Example Command Line

```
gls_event_hcs.bat 0 "D:\Illumina\HiSeqTemp\110616_SN450_0193_AB011Wacxx"
"\\hwisilon\service\1106\110616_SN450_0193_AB011Wacxx" "Paired End Indexing
Run"BeginRun HCS
```

Example Output

File name:

- event-BeginRun-12541247.txt

File contents:

```
cycleNumber = 0
runFolder = "D:\Illumina\HiSeqTemp\110616_SN450_0193_AB011Wacxx"
netFolder = "\\hw-isilon\service\1106\110616_SN450_0193_AB011Wacxx"
runType = "Paired End Indexing Run"
eventType = BeginRunsoftwareType = HCS
softwareType = HCS
```

EndRun Event

Source Data

RunInfo.xml

- [totalNumberOfCycles] - sum of NumCycles from <Reads> information gleaned from RunInfo.xml.

runParameters.xml

The following variables are found in the runParameters.xml file:

- <TempFolder>
- <OutputFolder>
- <RunID>

Pattern

```
gls_event_hcs_rta.bat [totalNumberOfCycles]
"<TempFolder>""<OutputFolder>\<RunID>" 1 EndRun HCS
```

Example Command Line

```
gls_event_hcs_rta.bat 209 "D:\Illumina\HiSeqTemp\110616_SN450_0193_
AB011Wacxx""\\hw-isilon\service\1106\110616_SN450_0193_AB011Wacxx" 1 EndRun HCS
```

Example Output

File name:

- event-EndRun-12555475.txt

File contents:

```
cycleNumber = 209
```

```
runFolder = "D:\Illumina\HiSeqTemp\110616_SN450_0193_AB011Wacxx"
netFolder = "\\hw-isilon\service\1106\110616_SN450_0193_AB011Wacxx"
readType = 1eventType = EndRun
softwareType = HCS
```

HiSeq/GAIIx v5 Sample Sheet Generation

This documentation has been updated and moved to the Illumina Integration Overview section. See *CASAVA 1.8.2 Sample Sheet Generation*.

Package Version: BaseSpace Clarity LIMS HiSeq/GAIIx Package v5.1 or later

Compatibility: BaseSpace Clarity LIMS v3.0 or later; NGS Extensions Package v5.0 or later. Recent features are marked with the package version in which they first appear.

New in HiSeq 5.1: -useSampleLimsID (-s) and -appendLimsID (-a) options.

Overview

The createSampleSheet script creates sample sheets for Illumina HiSeq and GAII instruments that are compatible with CASAVA 1.8.2.

Typically, sample sheet generation is run on the Cluster Generation step and the script is capable of creating one sample sheet per flow cell loaded during the step.

Installation and Upgrade Notes

To take advantage of the new features BaseSpace Clarity LIMS HiSeq/GAIIx Package v5.1 provides, customers who are installing or upgrading to the 5.1 package must manually modify the Cluster Generation (Illumina SBS) 5.0 process type configuration using the Operations Interface.

New features included in the 5.1 package enable the following:

- An option for populating the sample sheet SampleID field with the sample LIMS ID instead of name.
- An option for populating the sample sheet SampleID field with the protocol step LIMS ID appended to the end of the value entered. This option will ensure unique FASTQ file names per sequencing run when these are generated by Illumina.

Configuration

To enable sample sheet generation for multiple flow cells:

- The Cluster Generation step must be configured to produce one shared result file per sample sheet that will be created (see *Support for Multiple Sample Sheets* on page 2376).
- The EPP command line must specify the -c parameter with the LIMSID of the shared result file that will store each sample sheet in BaseSpace Clarity LIMS. The sample sheets will be attached to these result files and named as per the corresponding flow cell.

To enable unique FASTQ file names per sequencing run:

- The Cluster Generation step EPP command line must be configured to use the -useSampleLimsID (-s) and -appendLimsID (-a) options. See *Script Parameters and Usage* on page 2375
  - useSampleLimsID ensures unique entries in the SampleID column by using the sample LIMS ID instead of its name
  - appendLimsID ensures unique names per run by appending the LIMS ID of the current protocol step

Submitted Sample UDFs

Field Name	Field Type	Required?	Notes
Reference Genome	Text	No	Supplies data for sampleRef column in sample sheet.

Script Parameters and Usage

The following table defines the parameters used by the createSampleSheet script.

-u	LIMS username (Required)
-p	LIMS password (Required)
-i	
-c, -csvFileLimsIds	Sample sheet CSV file LIMS ID (Required - repeats allowed)
-e, -errorLogFileName	Log file name (required)
-l, -useProjectLimsID	Accepted values: true or false. Provide with quotes e.g. -l 'true' (Optional) Project LIMS ID will be used instead of the project name in the sample sheet (Optional) (lower case L)
-s, -useSampleLimsID	Accepted values: true or false . Provide with quotes e.g. -s 'true' (Optional) Sample LIMS IDs will be used instead of Sample Names in the sample sheet *(HiSeq/GAIIx 5.1)*
- a, -appendLimsID	Accepted values: true or false . Provide with quotes e.g. -a 'true' (Optional) The LIMS ID of the protocol step will be appended to the Sample Name values in the sample sheet. Use this option to guarantee unique FASTQ file names per run. *(HiSeq/GAIIx 5.1)*

Below is the default command line that ships with the Cluster Generation (Illumina SBS) 5.0 step in new installations. The createSampleSheet portion of the parameter string is shown in bold.

```
bash -c "/usr/bin/java -jar /opt/gls/clarity/extensions/Illumina_
HiSeq/v5/EPP/hiseqgaii-extensions.jar -u {username} -p {password} -i {processURI:v2:http}
script:setUDF -f 'Progress' -t '//input/@uri->//sample/@uri' -v 'Library ready
for sequencing' script:createSampleSheet -c {compoundOutputFileLuid1} -c
```


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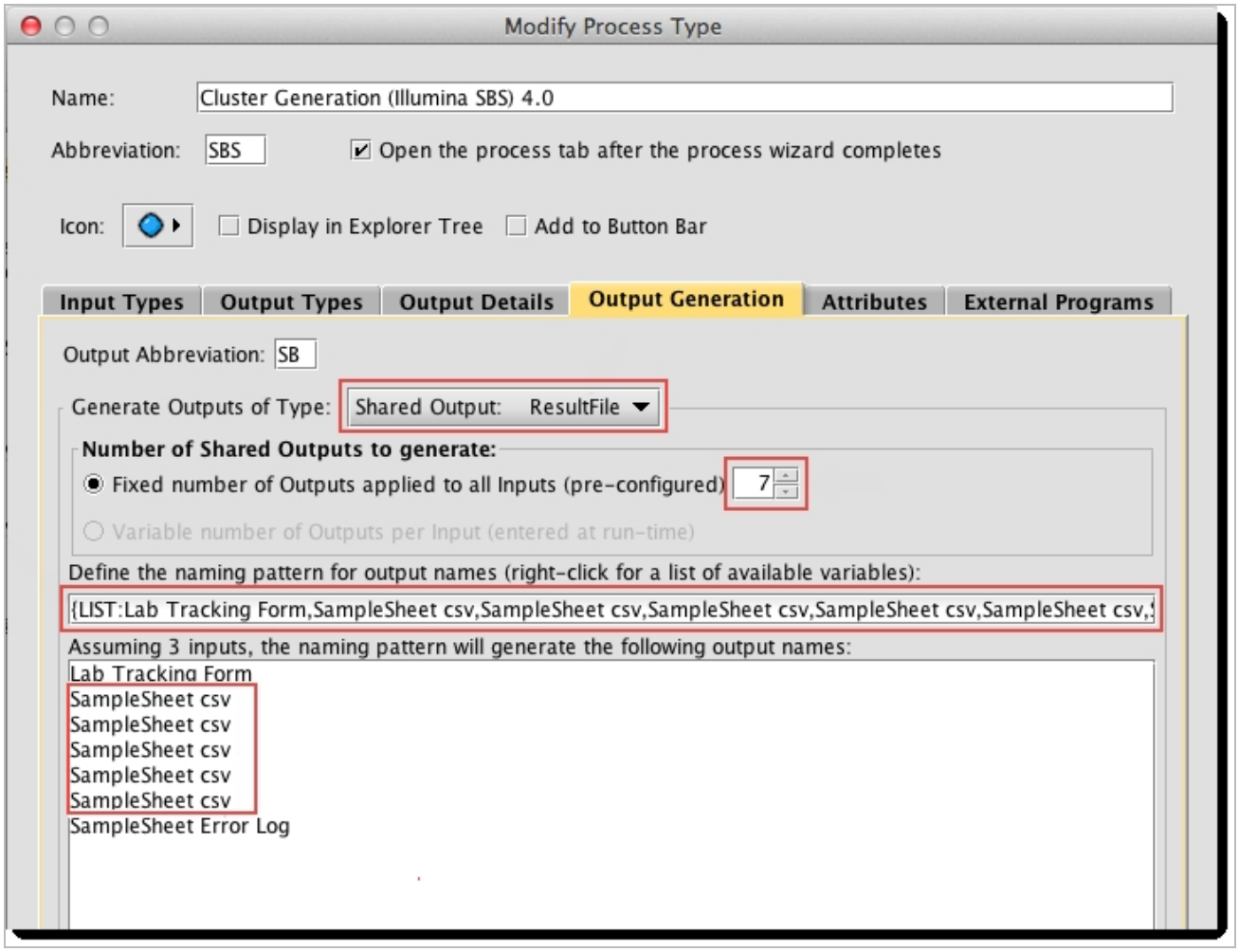
```
{compoundOutputFileLuid2} -c {compoundOutputFileLuid3} -c {compoundOutputFileLuid4} -c
{compoundOutputFileLuid5} -e {compoundOutputFileLuid6} script:labelNonLabeledOutputs -
l 'NoIndex' script:initArtifactUDFs"
```

Support for Container Types

Any one-dimensional container types with both numeric rows and numeric columns are supported.

Support for Multiple Sample Sheets

If generating multiple sample sheets, you must update the process to create placeholders for each of the sample sheets, as illustrated below.



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Logging

Info Messages	Reason/Condition
[Info] – Added data line for input: {input name}	For each line of data added to the sample sheet.
[Info] – Successfully generated Illumina HiSeq Sample Sheet.	On successful completion with no warnings or errors.

If any of the errors described in the following table are encountered:

- The script will terminate.
- An error log file will be written and attached to the step
- No sample sheet will be generated.

Error Messages	Reason/Condition
Unable to determine {user or password}	Unable to determine FTP credentials for ftp.user or ftp.password.
There are {number of flow cells created by the step} Flow Cell(s), but this step only supports sample sheet creation for {number of -c parameters pass on the command line} Flow Cell(s). Please restart the step with fewer samples.	More flow cells were found in the step than result file outputs in which to save the sample sheets. This can be remedied by changing the step configuration to accommodate additional files or by running the step with fewer inputs.
Container type not supported {containerType name} for input {input name}. The expected container type must be a single column or single row Flow Cell.	Unsupported container type used as a flow cell. Will occur if container has two dimensions.
Well coord not supported {input node xml} for input {input name}. The expected format is [numeric]:[numeric].	Unsupported well format for container type used as a flow cell.
Invalid number format for {placement string} for input {input name}. The expected format is [numeric]:[numeric].	Unexpected error caught trying to parse the well coordinate. Ensure the flow cell has numeric wells and numeric columns.
Unable to find reagent type {reagent label on input} for input {input name}.	There is no reagent type configured with the name of the reagent label found on the artifact.
Unable to find Index special type with Sequence attribute for input {input name}. Please check Reagent Type configuration of {reagent label on input}.	There is no reagent type configured with a special type of Index that has a Sequence attribute.

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Sample Sheet Data

- The contents of the sample sheet are ordered by Lane, then by Description (LIMS ID).
- Project and sample names in the sample sheet cannot contain illegal characters that are not allowed by some file systems. These characters are the space character and the following: ? ( ) [ ] / \ = + < > : ; " ' , * ^ | &
- The script will replace these characters with an underscore "_".

Column Header	Description
FCID	The name of the flow cell container.
Lane	The flow cell lane number corresponding to the input placement.
SampleID	Information from the submitted sample of the input, or the submitted sample of the (first) upstream pooling input if pooling exists. Will be sample name or LIMS ID, depending on command-line value. An additional command line option allows the step LIMS ID to be appended to the end of this value (e.g., "Sample1-1234"). See Script parameters and usage.
SampleRef	The value of the Reference Genome UDF on the input's sample.
Index	Uses the “Sequence” attribute value from index reagents.
Description	The LIMS ID of the step input, or the LIMS ID of the upstream pooling input if pooling exists.
Control	An input is considered a control if its name contains PhiX (case-sensitive).
Recipe	This column is always blank.
Operator	The name of the technician who initiated the step in the LIMS.
SampleProject	The project name of the Illumina Sequencing Analysis process input, or the project name of the upstream pooling input if pooling exists. If the '-l' parameter is true, the project LIMS ID will be used instead of the project name.

HiSeq/GAIIx v4 Sample Sheet Generation

Package Version: BaseSpace Clarity LIMS HiSeq/GAIIx Package v4.2, GenoLogics LIMS Illumina HiSeq/GAIIx Package v3.0.6

Overview

The createSampleSheet script creates sample sheets for Illumina HiSeq and GAII instruments that are compatible with CASAVA 1.8.2.

Typically, sample sheet generation is run on the Cluster Generation step and the script is capable of creating one sample sheet per flow cell loaded during the step.

Upgrade Notes

To take advantage of the new features BaseSpace Clarity LIMS HiSeq/GAIIx Package v4.2 provides, customers who are upgrading from a previous version must manually modify the Cluster Generation (Illumina SBS) 4.0 process type configuration using the Operations Interface.

New features included in v4.2 enable the following:

- Support for Illumina Rapid Flow Cells.
- Support for multiple (>1) flow cells per Cluster Generation step. Note that an additional shared result file output and EPP "-c {compoundOutputFileLuid#}" parameter must be added to the process for each additional flow cell to be supported.
- Using project LIMS IDs instead of project Names in sample sheets.
- The createSampleSheet -f parameter is no longer required and should be removed from the EPP command string. Otherwise, any script commands that follow it in the string will be ignored.

Configuration

To enable sample sheet generation for multiple flow cells:

- The Cluster Generation step must be configured to output one shared result file for every sample sheet that will be created.
- The EPP command line must specify the -c parameter with the LIMSID of the shared result file that will store each sample sheet in the LIMS. The sample sheets will be attached to these result files and named as per the corresponding flow cell.

Submitted Sample UDFs

Field Name	Field Type	Required?	Notes
Reference Genome	Text	No	Supplies data for sampleRef column in sample sheet.

Script Parameters

The following table defines the parameters used by the createSampleSheet script.

-u {username}	LIMS username (Required)
-p {password}	LIMS password (Required)
-i {URI}	LIMS process URI (Required) (lower case l)
-c, {csvFileLimsID}	Sample Sheet CSV file LIMS ID (Required - repeats allowed)

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-u {username}	LIMS username (Required)
-e, {errorLogFileName}	Error log file name (required)
-l, {useProjectLimsID}	Accepted values: true or false. P rovide with quotes e.g. -l 'true' (Optional) Project LIMS ID will be used instead of the project name in the sample sheet (lower case L)

Script Usage

Below is the default command line that ships with the Cluster Generation (Illumina SBS) 4.0 step in new installations. The createSampleSheet portion of the parameter string is shown in bold.

```
bash -c "/usr/bin/java -jar /opt/gls/clarity/extensions/Illumina_
HiSeq/v4/EPP/hiseqgaii-extensions.jar -u {username} -p {password} -i
{processURI:v2:http} script:setUDF -f 'Progress' -t '//input/@uri->//sample/@uri'
-v 'Library ready for sequencing' script:createSampleSheet -c {compoundOutputFileLuid1}
-c {compoundOutputFileLuid2} -c {compoundOutputFileLuid3} -c {compoundOutputFileLuid4} -c
{compoundOutputFileLuid5} -e {compoundOutputFileLuid6} script:labelNonLabeledOutputs -
l 'NoIndex' script:initArtifactUDFs"
```

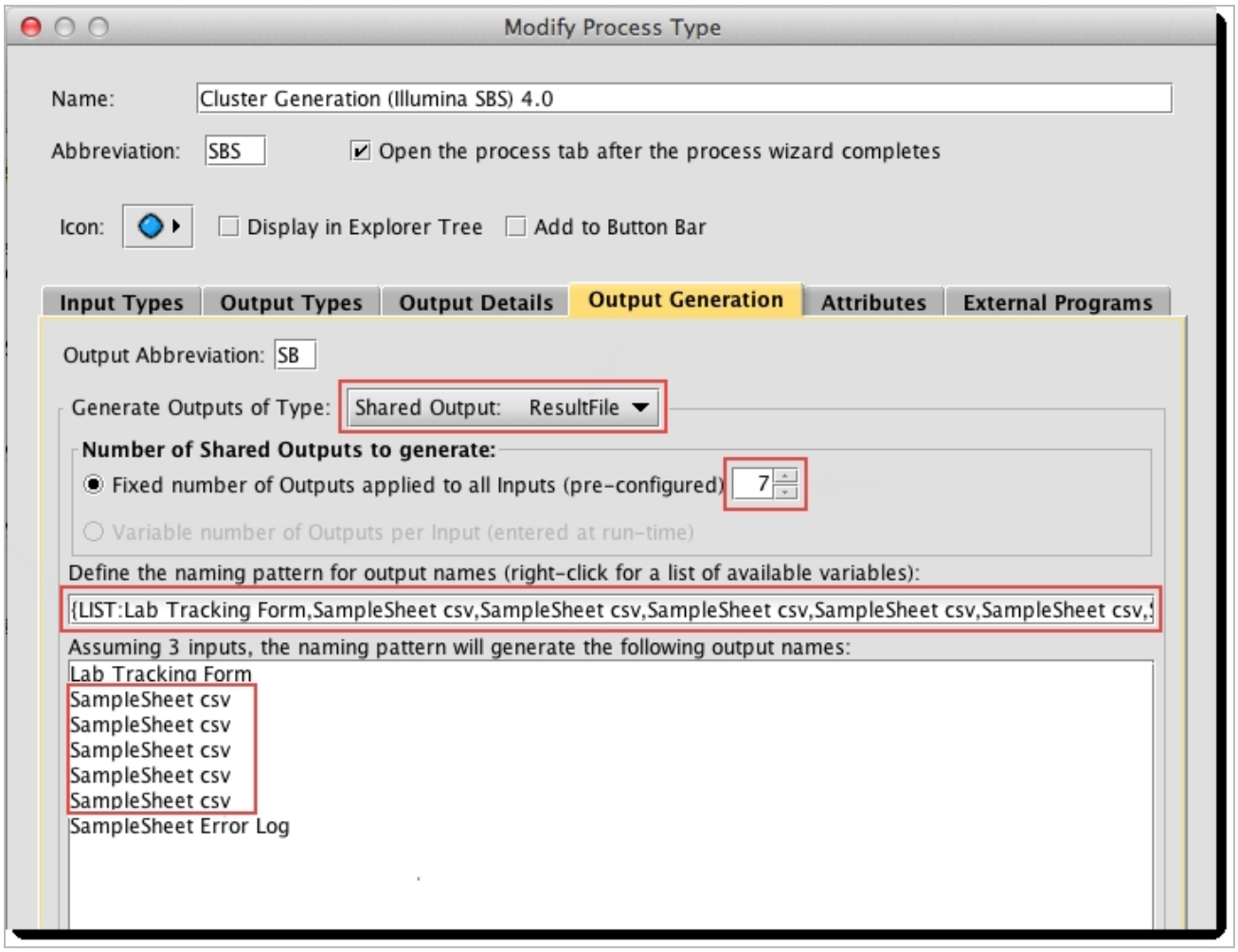
Support for Container Types

Any one dimensional container types with both numeric rows and numeric columns are supported.

Support for Multiple Sample Sheets

If generating multiple samplesheets, make sure that you update the process to create placeholders for each of the samplesheets as demonstrated in this screenshot:

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Logging

Info Messages	Reason/Condition
[Info] – Added data line for input: {input name}	A single line is logged for each data row added a sample sheet.
[Info] – Successfully generated Illumina HiSeq Sample Sheet.	On successful completion of the script following info message will be logged and all info messages will be written to the log file and attached back to the step in the LIMS.

When any of the following errors are encountered, the script will terminate, an error log file will be written and attached to the step. No sample sheet will be generated.

Error Messages	Reason/Condition
Unable to determine {user or password}	Unable to determine FTP credentials for ftp.user or ftp.password.

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Error Messages	Reason/Condition
There are {number of flow cells created by the step} flow cell(s), but a maximum of {number of -c parameters passed on the command line} sample sheet(s) can be created by this step. Please restart the step with fewer samples.	More flow cells than result files in which to save the sample sheets. This can be remedied by changing the configuration or running the step with less inputs.
Container type not supported {containerType name} for input {input name}. The expected container type must be a single column or single row Flow Cell.	Unsupported container type used as a flow cell. Will occur if container has two dimensions.
Well coord not supported {input node xml} for input {input name}. The expected format is [numeric]:[numeric].	Unsupported well format for container type used as a flow cell.
Invalid number format for {placement string} for input {input name}. The expected format is [numeric]:[numeric].	Unexpected error caught trying to parse the well coordinate. Ensure the flow cell has numeric wells and numeric columns.
Unable to find reagent type {reagent label on input} for input {input name}.	There is no reagent type configured with the name of the reagent label found on the artifact.
Unable to find Index special type with Sequence attribute for input {input name}. Please check Reagent Type configuration of {reagent label on input}.	There is no reagent type configured with a special type of Index that has a Sequence attribute.

Sample Sheet Data

- The contents of the sample sheet are ordered by Lane, then by Description (LIMS ID).
- Project and sample names in the sample sheet cannot contain illegal characters not allowed by some file systems. The characters not allowed are the space character and the following: ? ( ) [ ] / \ = + < > : ; " ' , * ^ | and
- The script will replace these characters with an underscore "_".

Column Header	Description
FCID	The name of the Flow Cell container.
Lane	The Flow Cell lane number corresponding to the input placement.
SampleID	The Submitted Sample name of the input, or the Submitted Sample name of the (first) upstream Pooling input if Pooling exists.
SampleRef	The value of the Reference Genome UDF on the input's Sample.
Index	Uses the “Sequence” attribute value from Index Reagents.

Column Header	Description
Description	The LIMS ID of the step input, or the LIMS ID of the upstream Pooling input if Pooling exists.
Control	An input is considered a control if its name contains PhiX (case-sensitive).
Recipe	This column is always blank.
Operator	The name of the technician who initiated the step in the LIMS.
SampleProject	The project name of the Illumina Sequencing Analysis process input, or the project name of the upstream Pooling input if Pooling exists. If the '-l' parameter is true, the project LIMS ID will be used instead of the project name.

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Clarity LIMS Product Analytics Service Integration

The documents in this section support the integration of the Clarity LIMS Product Analytics service. This integration supports on premise and Illumina cloud hosted deployments of Clarity LIMS v6.0 and later. Illumina Preset Protocols v2.3 or later is required for automation.

Supported Workflows

Clarity LIMS Product Analytics supports the workflows listed in the following table.

All workflows are provided as a configuration slice outside of the IPP installation.

Table 483 Supported Workflows

Software/Workflow	Version
ClarityLIMS-App	v6.0 and later[1]
Illumina-Preset-Protocols	v2.3 and later
NovaSeq X Series workflow	v1.0[2], v1.1[2], and v1.2[3]
NovaSeq 6000 v3 workflow	v3.4[4], v3.6[4], v3.8, and later
NextSeq 1000/2000 workflow	v2.2[4], v2.3[5], v2.4 and later[6]
Library Prep Validation	v2.0.5[4], v2.3.1, and later

[1] The integration supports on-premise or hosted installations. The on-premise support is only available to the NovaSeq 6000 v3 workflow. The NextSeq 1000/2000 and NovaSeq X Series workflows support only hosted installations. Clarity LIMS v6.2 discontinues support for CentOS and instead supports Oracle Linux as the superseding operating system.
[2] The NovaSeq X Series v1.0 and v1.1 integrations is only compatible with BaseSpaceLIMS-unified-product-analytics-scripts v1.2.0.
[3] The NovaSeq X Series v1.2 integration requires BaseSpaceLIMS-unified-product-analytics-scripts v1.2.1 and later.
[4] The CLPA enabled workflows are provided as separate configuration slices outside of the IPP installation.
[5] Requires CLPA v1.2 or earlier.
[6] Requires CLPA v1.3 or later due to updates to the `nextseq1k2k_analysisrun_completed.groovy` script.

Automation and Workflow Customization

Clarity LIMS Product Analytics (CLPA) integrates Clarity LIMS data and makes it available through the ICA CLPA service. CLPA allows you to aggregate and analyze lab data from the end-to-end workflow in a single location. This data is uploaded through the ICA LINK service. An ICA subscription is required in

order to enable the CLPA service.

CLPA supports the following workflows:

- NovaSeq 6000 v3
- NextSeq 1000/2000
- NovaSeq X Series
- Library Prep Validation

Other workflows and automations can be customized to add CLPA integration capabilities.

Prerequisites

Before customizing automation and workflows in Clarity LIMS Product Analytics, make sure that the following items are installed and configured:

- `BaseSpaceLIMS-unified-product-analytics`
- `BaseSpaceLIMS-unified-product-analytics-scripts`
- IPP v2.3 and later (with at least one supported workflow)
- Illumina Universal Sample Identifier custom field

Universal Sample Identifier

The Universal Sample Identifier is a Submitted Sample custom field used by the Register Sample automation. The field assigns a unique identifier to a sample across Illumina workflows. This identifier is used by CLPA to associate the sample with the workflows and analysis that result from a sequencing run and analysis (for example, sample lineage and traceability). Enter the Universal Sample Identifier value during sample accessioning. If the identifier is not provided during sample accessioning, the Register Sample automation does not have access to this value at run time. The CLPA association between the samples and other analytics data is also restricted.

When you install any of the CLPA enabled workflows, the Illumina Universal Sample Identifier custom field is automatically created. If the CLPA enabled workflows are not installed, the custom field must be created manually. When using the Illumina Universal Sample Identifier, the recommended data format is `usi.<clarity_hostname>.<sampleId>`.

For more information on installing CLPA enabled workflows, contact Illumina Support.

Add the Illumina Universal Sample Identifier Submitted Sample custom field.

1. On the main menu, select Configuration, and then select the Custom Fields tab.
2. On the Global Fields tab, in the Submitted Sample list, select the plus icon to create a custom field.
3. Enter Illumina Universal Sample Identifier as the field name.
4. Set the following properties:
    a. For Field Type, select Text.
    b. For Required Field, select No.

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c. For Read Only, select No.

5. Select Save.

Use Common CLPA Automation

Common CLPA automation scripts are used for workflow customization. Workflow-specific automations require specific custom fields from Illumina workflows.

The following automations can be used on any step:

- Step Started:

```
bash -l -c "/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/unified-product-analytics/automation/unified-
product-analytics-automation.jar script:executeUPAAutomationScript -i
{stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s
'com/illumina/upa/scripts/common/step_started.groovy'"
```

- Step Completed:

```
bash -l -c "/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/unified-product-analytics/automation/unified-
product-analytics-automation.jar script:executeUPAAutomationScript -i
{stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s
'com/illumina/upa/scripts/common/step_completed.groovy'"
```

The Register Sample automation can be used on the Library Prep step. If there are multiple steps in the Library Prep workflow, only add the Register Sample automation to the first step of the protocol. The script is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-
product-analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/common/register_
sample.groovy'
```

The Register Pool automation is available for the Pooling workflow. The script is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-
product-analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/pooling.groovy'
```

Each automation can be configured to log messages in a log file defined with the optional parameter -l. This parameter must be updated to refer to a correct log file placeholder (for example, {compoundOutputFileLuid1}), as different steps can have different log file placeholder positions.

Workflow Customization

Customize the workflow with CLPA automation as follows.

1. Identify the type of CLPA automation to be added along with the trigger location and style.
   For more information on CLPA automation types and trigger location and styles, refer to *Automation Scripts* for the applicable CLPA version.
2. Identify the protocol step to be customized.
3. Check for any existing automations on the triggers (for example, Step/Automatic upon entry or Step/Automatic upon exit). If there is an existing automation, modify the script as follows.
   a. Append the automation after the existing script with `&&`. Refer to the following examples.
      - Before append:
        ```
        bash -l -c "/opt/gls/clarity/bin/java -jar
        /opt/gls/clarity/extensions/<existingScript.jar>"
        ```
      - After append:
        ```
        bash -l -c "/opt/gls/clarity/bin/java -jar
        /opt/gls/clarity/extensions/<existingScript.jar> \
        && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-
        product-analytics/automation/unified-product-analytics-automation.jar \
        script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p
        {password} \
        -l {compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/common/step_
        completed.groovy'"
        ```

   When modifying an existing script, make sure to append the automation using the correct quotation marks (`"`). If you are appending an existing script on the next line, make sure that a backslash (`\`) is added on the last line of the script.

   b. Update the -l parameter to identify the correct Log File placeholder location. {compoundOutputFileLuid0} in the example refers to the first file placeholder.
   c. If necessary, update the automation name to describe the new behavior.
   d. In the Automation Use section, enable the step.
   e. Save the automation.
4. If there is not an existing automation on the protocol step, check for an existing automation that has been configured as follows.
   a. Identify the automation that is being used (for example, Register Step Started).
   b. Make sure that the log file setting for the existing script has the -l parameter. This parameter identifies the same Log File placeholder that is used with the step.
      - If the Log File location is the same, enable the step in the Automation Use section.
      - If the Log File location is not the same, create a separate automation and enable the step in the Automation Use section of the new automation.
   c. Save the automation.
5. Go to Configuration and select Lab Work.

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6. Select the step and configure the Trigger Location/Style. Refer to *Automation Scripts* for the applicable CLPA version.
   After the automation is enabled, the automation configuration option becomes available on the Step configuration page.
7. Save the step.

Clarity LIMS Product Analytics Integration v1.3 Configuration and User Interaction

The Clarity LIMS Product Analytics Integration v1.3 allows you to aggregate and analyze lab data from the workflow in a single location. Data is uploaded to the Illumina Connected Analytics (ICA) LINK service. A subscription to ICA is required to enable the CLPA service.

Clarity LIMS Product Analytics Integration v1.3 includes the following changes:

- The `step_completed.groovy` script no longer fails when there are zero outputs for a step, such as no output step and file placeholder.
- The `nextseq1k2k_analysisrun_completed.groovy` script removes the # of > = Q30 Bases (PF) and Mean Quality Score (PF) fields. These fields are no longer supported in the NextSeq 1000/2000 Sequencing v2.4 workflows.
- The configure_upa.sh script provides the default value during setup.
- Configurable properties are now in the database and can be updated with the omxProps-ConfigTool utility.

This integration also supports the following workflows:

- NovaSeq 6000 v3
- NextSeq 1000/2000
- NovaSeq X Series
- Library Prep Validation

Other workflows can be customized to add Clarity LIMS Product Analytics integration capabilities. For more information, refer to *Automation and Workflow Customization* on page 2384.

Supported Workflows

Clarity LIMS Product Analytics Integration v1.3 supports the applicable workflows identified in *Supported Workflows* on page 2384. The NextSeq 1000/2000 Sequencing v2.4 workflow and later requires this integration due to updates to the `nextseq1k2k_analysisrun_completed.groovy` script.

Components

Clarity LIMS Product Analytics Integration v1.3 includes the following required components:

- `BaseSpaceLIMS-unified-products-analytics`—The core service of the Clarity LIMS Product Analytics Integration. This service installs the daemon service that publishes messages to the product analytics through the LINK service.
- `BaseSpaceLIMS-unified-products-analytics-scripts`—The automation scripts of the Clarity LIMS Product Analytics Integration. If new workflows or new product analytics scripts are introduced, this component can be upgraded.

For more information on the Clarity LIMS Product Analytics Integration scripts, refer to *Automation Scripts* on page 2389.

Automation Scripts

The Clarity LIMS Product Analytics Integration v1.3 automation scripts are installed through the `BaseSpaceLIMS-unified-products-analytics-scripts` RPM. These scripts process Clarity LIMS data and prepare data for upload to the LINK service. There are two types of CLPA scripts: common and workflow-specific.

Common Clarity LIMS Product Analytics Scripts

Common CLPA scripts can be used with any compatible workflows. The following table lists and describes the common CLPA scripts.

Automation Name	Script Name	Trigger Location/Style	Trigger Style	Purpose	Notes
Register Step Started	`common/step_started.groovy`	Step	Automatic upon entry	Sends the step start time information to CLPA.	Can be used on any step.
Register Step Completed	`common/step_completed.groovy`	Step	Automatic upon exit	Sends the step completed time information to CLPA.	Can be used on any step.

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Automation Name	Script Name	Trigger Location/Style	Trigger Style	Purpose	Notes
Register Sample	`common/register_sample.groovy`	Step	Automatic upon entry	Sends sample information used by the lab workflow to CLPA.	Can be used on any step, but it is recommended that this script is enabled at the first step of the workflow. Only one Register Sample automation is required per workflow.
Register Pool	`common/pooling.groovy`	Step	Automatic upon exit	Sends pool and library information used by the lab workflow to CLPA.	Can be used on any pooling step. It is expected that libraries in the pool have been through the Add Labels step. Control samples do not need a label.

Workflow-Specific Clarity LIMS Product Analytics Scripts

Workflow-specific scripts work with Illumina workflows. These scripts require a specific set of custom fields and a specific protocol order in the workflow. The following table shows the workflow-specific scripts.

These scripts require custom fields to be available at their corresponding steps (listed under Clarity LIMS Product Analytics Automation Name and Remarks). Adding or enabling workflow-specific CLPA scripts on non-Illumina workflows is not supported. Data loss can occur.

As many of the scripts in this table share trigger location and style, they are combined into a single automation.

Automation Name	Script Name	Used In	Workflow Version	Trigger Location/ Style	Trigger Style	Purpose/ Notes
Register NextSeq1k2k Run Association	`nextseq1k2k/nextseq1k2k_associate_seqrun.groovy`	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing)	v2.2 v2.3 and later	Step	Automatic upon entry	Associates the sequencing run with the samples. This association links the Instrument Run ID with the sample IDs.
Register NextSeq1k2k RunStarted	`nextseq1k2k/nextseq1k2k_seqrun_started.groovy`	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing)	v2.2 v2.3 and later	Step	Automatic upon entry	Records the started time of the sequencing run.

Automation Name	Script Name	Used In	Workflow Version	Trigger Location/ Style	Trigger Style	Purpose/ Notes
Register NextSeq1k2k RunCompleted	`nextseq1k2k/nextseq1k2k_seqrun_completed.groovy`	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing)	v2.2 v2.3 and later	Step	Automatic upon exit	Records the following information: • Run completion time • Run status • Run metrics This script requires the Cloud Run ID custom field from the Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing) step.

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Automation Name	Script Name	Used In	Workflow Version	Trigger Location/ Style	Trigger Style	Purpose/ Notes
Register NextSeq1k2k Run	`nextseq1k2k/register_nextseq1k2k_run.groovy`	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing)	v2.2 v2.3 and later	Step	Automatic upon entry	Records the sequencing run configuration. This script requires all custom fields related to the run configuration in the Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing) step.

For Research Use Only. Not for use in diagnostic procedures.

Automation Name	Script Name	Used In	Workflow Version	Trigger Location/ Style	Trigger Style	Purpose/ Notes
Register Analysis Completed	`nextseq1k2k/nextseq1k2k_analysisrun_completed.groovy`	Demultiplexing (NextSeq 1000/2000 Sequencing)	v2.2 v2.3 and later	Step	Automatic upon exit	Records the NextSeq 1000/2000 analysis status and result. This script requires the fields related to Cloud Analysis Workflow Versions and Local Analysis Workflow Versions from the Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing) step.

For Research Use Only. Not for use in diagnostic procedures.

Automation Name	Script Name	Used In	Workflow Version	Trigger Location/ Style	Trigger Style	Purpose/ Notes
Register NovaSeq Run Association	`novaseq6000/novaseq_associate_seqrun.groovy`	AUTOMATED - NovaSeq Run (NovaSeq 6000)	v3.4 v3.6 v3.8 and later	Step	Automatic upon entry	Associates the sequencing run with the samples. This association links the Instrument Run ID with the sample IDs.
Register NovaSeq RunStarted	`novaseq6000/novaseq_seqrun_started.groovy`	AUTOMATED - NovaSeq Run (NovaSeq 6000)	v3.4 v3.6 v3.8 and later	Step	Automatic upon entry	Records the started time of the sequencing run.
Register NovaSeq RunCompleted	`novaseq6000/novaseq_seqrun_completed.groovy`	AUTOMATED - NovaSeq Run (NovaSeq 6000)	v3.4 v3.6 v3.8 and later	Step	Automatic upon exit	Records the following information: • Run completion time • Run status • Run metrics

Automation Name	Script Name	Used In	Workflow Version	Trigger Location/ Style	Trigger Style	Purpose/ Notes
Register NovaSeq Run	`novaseq6000/register_novaseq_run.groovy`	AUTOMATED - NovaSeq Run (NovaSeq 6000)	v3.4 v3.6 v3.8 and later	Step	Automatic upon entry	Records the sequencing run configuration. This script requires all custom fields related to the run configuration. For Standard workflow: • Dilute and Denature (NovaSeq 6000) For XP workflow: • Load to Flowcell (NovaSeq 6000)

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Automation Name	Script Name	Used In	Workflow Version	Trigger Location/ Style	Trigger Style	Purpose/ Notes
Register NovaSeq X Run Association	`novaseqxseries/novaseqxseries_associate_seqrun.groovy`	AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing)	v1.0 and later	Step	Automatic upon entry	Associates the sequencing run with the samples. This association links the Instrument Run ID with the sample IDs.
Register NovaSeq X RunStarted	`novaseqxseries/novaseqxseries_seqrun_started.groovy`	AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing)	v1.0 and later	Step	Automatic upon entry	Records the started time of the sequencing run.

For Research Use Only. Not for use in diagnostic procedures.

Automation Name	Script Name	Used In	Workflow Version	Trigger Location/ Style	Trigger Style	Purpose/ Notes
Register NovaSeq X RunCompleted	`novaseqxseries/novaseqxseries_seqrun_completed.groovy`	AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing)	v1.0 and later	Step	Automatic upon exit	Records the following information: • Run completion time • Run status • Run metrics This script also requires the Cloud Run ID custom field from the Load to Library Tube Strip (NovaSeq X Series Sequencing) step.

For Research Use Only. Not for use in diagnostic procedures.

Automation Name	Script Name	Used In	Workflow Version	Trigger Location/ Style	Trigger Style	Purpose/ Notes
Register NovaSeq X Run	`novaseqxseries/register_novaseqxseries_run.groovy`	AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing)	v1.0 and later	Step	Automatic upon entry	Records the sequencing run configuration. This script requires all custom fields related to the run configuration from the Load to Library Tube Strip (NovaSeq X Series Sequencing) step.

Clarity LIMS Product Analytics Integration v1.2 Configuration and User Interaction

The Clarity LIMS Product Analytics Integration v1.2 allows you to aggregate and analyze lab data from the workflow in a single location. The integration also integrates Clarity LIMS data into the Clarity LIMS Product Analytics (CLPA) service. Data are uploaded to the Illumina Connected Analytics(ICA) LINK service. A subscription to ICA is required to enable the CLPA service.

Clarity LIMS Product Analytics Integration v1.2 is used with Clarity LIMS v6.2 and later. The integration supports on-site and hosted installations of Clarity LIMS. Illumina Preset Protocols (IPP) v2.6 or later is required for automation.

Supported Workflows

Clarity LIMS Product Analytics Integration v1.2 supports the applicable workflows identified in *Supported Workflows* on page 2384.

Components

Clarity LIMS Product Analytics Integration v1.2 includes the following required components:

- BaseSpaceLIMS-unified-products-analytics—The core service of the Clarity LIMS Product Analytics Integration. This service installs the daemon service that publishes messages to the product analytics through the LINK service.
- BaseSpaceLIMS-unified-products-analytics-scripts—The automation scripts of the Clarity LIMS Product Analytics Integration. If new workflows or new product analytics scripts are introduced, this component can be upgraded.

For more information on the Clarity LIMS Product Analytics Integration scripts, refer to *Automation Scripts* on page 2400.

Automation Scripts

The Clarity LIMS Product Analytics Integration v1.2 automation scripts are installed through the `BaseSpaceLIMS-unified-products-analytics-scripts` RPM. These scripts process Clarity LIMS data and prepare data for upload to the LINK service. There are two types of CLPA scripts: common and workflow-specific.

Common Clarity LIMS Product Analytics Scripts

Common CLPA scripts can be used with any compatible workflows. The following table lists and describes the common CLPA scripts.

Automation Name	Script Name	Trigger Location/Style	Trigger Style	Purpose	Notes
Register Step Started	`common/step_started.groovy`	Step	Automatic upon entry	Saves information on the step start time.	Can be used on any step.

For Research Use Only. Not for use in diagnostic procedures.

Automation Name	Script Name	Trigger Location/Style	Trigger Style	Purpose	Notes
Register Step Completed	`common/step_completed.groovy`	Step	Automatic upon exit	Saves information on the step-completed time.	Can be used on any step.
Register Sample	`common/register_sample.groovy`	Step	Automatic upon entry	Saves sample information used by the lab workflow.	Can be used on any step. It is recommended that this script is enabled at the first step of the workflow. Only one is required per workflow. This script requires the Submitted Sample field named Illumina Universal Sample Identifier.

For Research Use Only. Not for use in diagnostic procedures.

Automation Name	Script Name	Trigger Location/Style	Trigger Style	Purpose	Notes
Register Pool	`common/pooling.groovy`	Step	Automatic upon exit	Saves pool and library information used by the Clarity LIMS workflow.	Can be used on any pooling step. it is expected that libraries in the pool have been through the Add Labels step. Control samples do not need a label.

Workflow-Specific Clarity LIMS Product Analytics Scripts

Workflow-specific scripts work with Illumina workflows. These scripts require a specific set of custom fields and a specific protocol order in the workflow. The following table shows the workflow-specific scripts.

These scripts require custom fields to be available at their corresponding steps (listed under Clarity LIMS Product Analytics Automation Name and Remarks). Adding or enabling workflow-specific CLPA scripts on non-Illumina workflows is not supported. Data loss can occur.

As many of the scripts in this table share trigger location and style, they are combined into a single automation.

Automation Name	Script Name	Used In	Trigger Location/Style	Trigger Style	Purpose/Notes
Register NextSeq1k 2k Run Association	`nextseq1k2k/nextseq1k2k_associate_seqrun.groovy`	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.2) AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3)	Step	Automatic upon entry	Associates the sequencing run with the samples. This association links the Instrument Run ID with the sample IDs.
Register NextSeq1k 2k RunStarted	`nextseq1k2k/nextseq1k2k_seqrun_started.groovy`	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.2) AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3)	Step	Automatic upon entry	Records the started time of the sequencing run.

Automation Name	Script Name	Used In	Trigger Location/Style	Trigger Style	Purpose/Notes
Register NextSeq1k2k RunCompleted	`nextseq1k2k/nextseq1k2k_seqrun_completed.groovy`	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.2) AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3)	Step	Automatic upon exit	Records the following information: • Run completion time • Run status • Run metrics This script requires the Cloud Run ID custom field from the Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.2) and Load to Reagent (NextSeq 1000/2000 Sequencing v2.3) steps.

For Research Use Only. Not for use in diagnostic procedures.

Automation Name	Script Name	Used In	Trigger Location/Style	Trigger Style	Purpose/Notes
Register NextSeq1k2k Run	`nextseq1k2k/register_nextseq1k2k_run.groovy`	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.2) AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.3)	Step	Automatic upon entry	Records the sequencing run configuration. This script requires all custom fields related to the run configuration in the Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.2) and Load to Reagent (NextSeq 1000/2000 Sequencing v2.3) steps.

For Research Use Only. Not for use in diagnostic procedures.

Automation Name	Script Name	Used In	Trigger Location/Style	Trigger Style	Purpose/Notes
Register Analysis Completed	`nextseq1k2k/nextseq1k2k_analysisrun_completed.groovy`	Demultiplexing (NextSeq 1000/2000 Sequencing v2.2) Demultiplexing (NextSeq 1000/2000 Sequencing v2.3)	Step	Automatic upon exit	Records the NextSeq1k 2k analysis status and result. This script requires the Cloud Analysis Workflow Versions and Local Analysis Workflow Versions custom fields from the Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.2) and Load to Reagent (NextSeq 1000/2000 Sequencing v2.3) steps.

For Research Use Only. Not for use in diagnostic procedures.

Automation Name	Script Name	Used In	Trigger Location/Style	Trigger Style	Purpose/Notes
Register NovaSeq Run Association	`novaseq6000/novaseq_associate_seqrun.groovy`	AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.4) AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.6) AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8)	Step	Automatic upon entry	Associates the sequencing run with the samples. This association links the Instrument Run ID with the sample IDs.
Register NovaSeq RunStarted	`novaseq6000/novaseq_seqrun_started.groovy`	AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.4) AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.6) AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8)	Step	Automatic upon entry	Records the started time of the sequencing run.

For Research Use Only. Not for use in diagnostic procedures.

Automation Name	Script Name	Used In	Trigger Location/Style	Trigger Style	Purpose/Notes
Register NovaSeq RunCompleted	`novaseq6000/novaseq_seqrun_completed.groovy`	AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.4) AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.6) AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8)	Step	Automatic upon exit	Records the following information: • Run completion time • Run status • Run metrics

For Research Use Only. Not for use in diagnostic procedures.

Automation Name	Script Name	Used In	Trigger Location/Style	Trigger Style	Purpose/Notes
Register NovaSeq Run	`novaseq6000/register_novaseq_run.groovy`	AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.4) AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.6) AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8)	Step	Automatic upon entry	Records the sequencing run configuration. This script requires all custom fields related to the run configuration. For Standard workflow: • Dilute and Denature (NovaSeq 6000 v3.4) • Dilute and Denature (NovaSeq 6000 v3.6) • Dilute and Denature (NovaSeq 6000 v3.8) For XP workflow: • Load to Flow Cell (NovaSeq 6000 v3.4) • Load to Flow Cell (NovaSe

Automation Name	Script Name	Used In	Trigger Location/Style	Trigger Style	Purpose/Notes
Register NovaSeq X Run Association	`novaseqxseries/novaseqxseries_associate_seqrun.groovy`	AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0)	Step	Automatic upon entry	Associates the sequencing run with the samples. This association links the Instrument Run ID with the sample IDs.
Register NovaSeq X RunStarted	`novaseqxseries/novaseqxseries_seqrun_started.groovy`	AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0)	Step	Automatic upon entry	Records the started time of the sequencing run.

For Research Use Only. Not for use in diagnostic procedures.

Automation Name	Script Name	Used In	Trigger Location/Style	Trigger Style	Purpose/Notes
Register NovaSeq X RunCompleted	`novaseqxseries/novaseqxseries_seqrun_completed.groovy`	AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0)	Step	Automatic upon exit	Records the following information: • Run completion time • Run status • Run metrics This script also requires the Cloud Run ID custom field from the Load to Library Tube Strip (NovaSeq X Series Sequencing v1.0) step.

For Research Use Only. Not for use in diagnostic procedures.

Automation Name	Script Name	Used In	Trigger Location/Style	Trigger Style	Purpose/Notes
Register NovaSeq X Run	`novaseqxseries/register_novaseqxseries_run.groovy`	AUTOMATED - Sequencing Run (NovaSeq X Series Sequencing v1.0)	Step	Automatic upon entry	Records the sequencing run configuration. This script requires all custom fields related to the run configuration from the Load to Library Tube Strip (NovaSeq X Series Sequencing v1.0) step.

Automation Customization

Before customizing the automations in Clarity LIMS Product Analytics Integration v1.2, make sure that the following items are installed and configured:

- `BaseSpaceLIMS-unified-product-analytics`
- `BaseSpaceLIMS-unified-product-analytics-scripts`
- IPP v2.3 and later (with at least one supported workflow)

Universal Sample Identifier

The Universal Sample Identifier is a Submitted Sample custom field used by the Register Sample automation. The field assigns a unique identifier to a sample across Illumina workflows. This identifier is used by CLPA to associate the sample with the workflows and analysis that result from a sequencing run and analysis (eg, sample lineage and traceability). Enter the Universal Sample Identifier value during sample accessioning. If the identifier is not provided during sample accessioning, the Register Sample automation does not have access to this value at run time. The CLPA association between the samples

and other analytics data is also restricted.

When you install any of the CLPA enabled workflows, the Illumina Universal Sample Identifier custom field is automatically created. If the CLPA enabled workflows are not installed, the custom field must be created manually. When using the Illumina Universal Sample Identifier, the recommended data format is `usi.<clarity_hostname>.<sampleId>`.

For more information on installing CLPA enabled workflows, contact Illumina Support.

Add the Illumina Universal Sample Identifier Submitted Sample custom field.

1. On the main menu, select Configuration, and then select the Custom Fields tab.
2. On the Global Fields tab, in the Submitted Sample list, select the plus icon to create a custom field.
3. Enter Illumina Universal Sample Identifier as the field name.
4. Set the following properties:
    a. For Field Type, select Text.
    b. For Required Field, select No.
    c. For Read Only, select No.
5. Select Save.

Use Common CLPA Automation

Common CLPA automation scripts are used for workflow customization. Each of these automation scripts logs a message into a log file that is defined with the parameter -l. Because steps can have different log placeholder positions, you must update the -l parameter to reference the correct log file placeholder (eg, `{compoundOutputFileLuid1}`).

The following automations can be used on any step.

- Step Started:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_started.groovy'"
```

- Step Completed:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_completed.groovy'"
```

The Register Sample automation can be used on the Library Prep step. If there are multiple steps in the Library Prep workflow, only add the Register Sample automation to the first step of the protocol. The script is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-
product-analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/common/register_
sample.groovy'
```

The Register Pool automation is available for the Pooling workflow. The script is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-
product-analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/pooling.groovy'
```

Workflow Customization

Customize the workflow with CLPA automation as follows.

1. Identify the type of CLPA automation to be added along with the trigger location and style.
   For more information on CLPA automation types and trigger location and styles, refer to *Automation Scripts* on page 2400.
2. Identify the protocol step to be customized.
3. Check for any existing automations on the triggers (eg, Step/Automatic upon entry or Step/Automatic upon exit). If there is an existing automation, modify the script as follows.
   a. Append the automation after the existing script with `&&`. Refer to the following examples.

   > When modifying an existing script, make sure to append the automation using the correct quotation marks (`"`). If you are appending an existing script on the next line, make sure that a backslash (`\`) is added on the last line of the script.

   - Before append:

   ```
   bash -l -c "/opt/gls/clarity/bin/java -jar
   /opt/gls/clarity/extensions/<existingScript.jar>"
   ```

   - After append:

   ```
   bash -l -c "/opt/gls/clarity/bin/java -jar
   /opt/gls/clarity/extensions/<existingScript.jar> \
   && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
   analytics/automation/unified-product-analytics-automation.jar \
   script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password}
   \
   -l {compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/common/step_
   completed.groovy'"
   ```

   b. Update the -l parameter to identify the correct Log File placeholder location. {compoundOutputFileLuid0} in the example refers to the first file placeholder.
   c. If necessary, update the automation name to describe the new behavior.

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d. In the Automation Use section, enable the step.

e. Save the automation.

4. If there is not an existing automation on the protocol step, check for an existing automation that has been configured as follows.

   a. Identify the automation that is being used (eg, Register Step Started).

   b. Make sure that the log file setting for the existing script has the -l parameter. This parameter identifies the same Log File placeholder that is used with the step.

      - If the Log File location is the same, enable the step in the Automation Use section.
      - If the Log File location is not the same, create a separate automation and enable the step in the Automation Use section of the new automation.

   c. Save the automation.

5. Go to Configuration and select Lab Work.

6. Select the step and configure the Trigger Location/Style. Refer to *Automation Scripts* on page 2400.
   After the automation is enabled, the automation configuration option becomes available on the Step configuration page.

7. Save the step.

Clarity LIMS Product Analytics Integration v1.1 Configuration and User Interaction

The Clarity LIMS Product Analytics Integration v1.1 allows you to aggregate and analyze lab data from the workflow in a single location. The integration also integrates Clarity LIMS data into the Illumina Connected Analytics Product Analytics service. Data are uploaded to the ICA LINK service. A subscription to Illumina Connected Analytics(ICA) is required to enable the Clarity LIMS Product Analytics service.

Clarity LIMS Product Analytics Integration v1.1 is used with Clarity LIMS v6.0 and later. The integration supports on-site and hosted installations of Clarity LIMS. Illumina Preset Protocols (IPP) v2.3 or later is required for automation.

Supported Workflows

Clarity LIMS Product Analytics Integration v1.1 supports the applicable workflows identified in *Supported Workflows* on page 2384.

Components

Clarity LIMS Product Analytics Integration v1.1 includes the following required components:

- BaseSpaceLIMS-unified-products-analytics—The core service of the Clarity LIMS Product Analytics Integration. This service installs the daemon service that publishes messages to the product analytics through the LINK service.
- BaseSpaceLIMS-unified-products-analytics-scripts—The automation scripts of the Clarity LIMS Product Analytics Integration. If new workflows or new product analytics scripts are introduced, this component can be upgraded.

For more information on the Clarity LIMS Product Analytics Integration scripts, refer to *Automation Scripts* on page 2416.

Terminology

As of Clarity LIMS v5, the term user-defined field (UDF) has been replaced with custom field in the user interface. However, the API resource is still called UDF.

There are two types of custom fields:

- Master step fields—Configured on master steps. Master step fields only apply to the following:
  - The master step on which the fields are configured.
  - The steps derived from those master steps.
- Global fields—Configured on entities (eg, submitted sample, derived sample, measurement, etc.). Global fields apply to the entire Clarity LIMS system.

Automation Scripts

The Clarity LIMS Product Analytics Integration v1.1 automation scripts are installed through the `BaseSpaceLIMS-unified-products-analytics-scripts` RPM. These scripts process Clarity LIMS data and prepare data for upload to the LINK service. There are two types of CLPA scripts: common and workflow-specific.

Common CLPA Scripts

Common CLPA scripts can be used with any compatible workflows. The following table lists and describes the common CLPA scripts.

Automation Name	Script Name	Trigger Location/Style	Purpose	Notes
Register Step Started	`common/step_started.groovy`	Location—Step Style—Automatic upon entry	Saves information on the step start time.	Can be used on any step.

For Research Use Only. Not for use in diagnostic procedures.

Automation Name	Script Name	Trigger Location/Style	Purpose	Notes
Register Step Completed	`common/step_completed.groovy`	Location—Step Style—Automatic upon exit	Saves information on the step-completed time.	Can be used on any step.
Register Sample	`common/register_sample.groovy`	Location—Step Style—Automatic upon entry	Saves sample information used by the lab workflow.	Can be used on any step. It is recommended that this script is enabled at the first step of the workflow. Only one is required per workflow. This script requires the Submitted Sample field named Illumina Universal Sample Identifier.
Register Pool	`common/pooling.groovy`	Location—Step Style—Automatic upon exit	Saves pool and library information used by the Clarity LIMS workflow.	Can be used on any pooling step. it is expected that libraries in the pool have been through the Add Labels step. Control samples do not need a label.

For Research Use Only. Not for use in diagnostic procedures.

Workflow-Specific CLPA Scripts

Workflow-specific scripts work with Illumina workflows. These scripts require a specific set of custom fields and a specific protocol order in the workflow. The following table shows the workflow-specific scripts.

These scripts require custom fields to be available at their corresponding steps (listed under Clarity LIMS Product Analytics Automation Name and Remarks). Adding or enabling workflow-specific CLPA scripts on non-Illumina workflows is not supported. Data loss can occur.

As many of the scripts in this table share trigger location and style, they are combined into a single automation.

Automation Name	Script Name	Used In	Trigger Location/Style	Purpose/Notes
Register NextSeq1k2k Run Association	`nextseq1k2k/nextseq1k2k_associate_seqrun.groovy`	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.2)	Location—Step Style—Automatic upon entry	Associates the sequencing run with the samples. This association links the Instrument Run ID with the sample IDs.
Register NextSeq1k2k RunStarted	`nextseq1k2k/nextseq1k2k_seqrun_started.groovy`	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.2)	Location—Step Style—Automatic upon entry	Records the started time of the sequencing run.

Automation Name	Script Name	Used In	Trigger Location/Style	Purpose/Notes
Register NextSeq1k2k RunCompleted	`nextseq1k2k/nextseq1k2k_seqrun_completed.groovy`	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.2)	Location—Step Style—Automatic upon exit	Records the following information: • Run completion time • Run status • Run metrics This script requires the Cloud Run ID custom field from the Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.2) step.
Register NextSeq1k2k Run	`nextseq1k2k/register_nextseq1k2k_run.groovy`	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.2)	Location—Step Style—Automatic upon entry	Records the sequencing run configuration. This script requires all custom fields related to the run configuration in the Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.2) step.

For Research Use Only. Not for use in diagnostic procedures.

Automation Name	Script Name	Used In	Trigger Location/Style	Purpose/Notes
Register Analysis Completed	`nextseq1k2k/nextseq1k2k_analysisrun_completed.groovy`	Demultiplexing (NextSeq 1000/2000 Sequencing v2.2)	Location—Step Style—Automatic upon exit	Records the NextSeq1k2k analysis status and result. This script requires the Cloud Analysis Workflow Versions and Local Analysis Workflow Versions custom fields from the Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.2) step.
Register NovaSeq Run Association	`novaseq6000/novaseq_associate_seqrun.groovy`	AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.4) AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.6) AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8)	Location—Step Style—Automatic upon entry	Associates the sequencing run with the samples. This association links the Instrument Run ID with the sample IDs.

For Research Use Only. Not for use in diagnostic procedures.

Automation Name	Script Name	Used In	Trigger Location/Style	Purpose/Notes
Register NovaSeq RunStarted	`novaseq6000/novaseq_seqrun_started.groovy`	AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.4) AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.6) AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8)	Location—Step Style—Automatic upon entry	Records the started time of the sequencing run.
Register NovaSeq RunCompleted	`novaseq6000/novaseq_seqrun_completed.groovy`	AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.4) AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.6) AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8)	Location—Step Style—Automatic upon exit	Records the following information: • Run completion time • Run status • Run metrics

For Research Use Only. Not for use in diagnostic procedures.

Automation Name	Script Name	Used In	Trigger Location/Style	Purpose/Notes
Register NovaSeq Run	`novaseq6000/register_novaseq_run.groovy`	AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.4) AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.6) AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.8)	Location—Step Style—Automatic upon entry	Records the sequencing run configuration. This script requires all custom fields related to the run configuration. For Standard workflow: • Dilute and Denature (NovaSeq 6000 v3.4) • Dilute and Denature (NovaSeq 6000 v3.6) • Dilute and Denature (NovaSeq 6000 v3.8) For XP workflow: • Load to Flow Cell (NovaSeq 6000 v3.4) • Load to Flow Cell (NovaSeq 6000 v3.6) • Load to Flow Cell (NovaSeq 6000 v3.8)

For Research Use Only. Not for use in diagnostic procedures.

Automation Customization

Before customizing the automations in CLPA Integration v1.1, make sure that the following items are installed and configured:

- `BaseSpaceLIMS-unified-product-analytics`
- `BaseSpaceLIMS-unified-product-analytics-scripts`
- Illumina Preset Protocols (IPP) v2.3 and later (with at least one supported workflow)

Universal Sample Identifier

The Universal Sample Identifier is a Submitted Sample custom field used by the Register Sample automation. The field assigns a unique identifier to a sample across Illumina workflows. This identifier is used by CLPA to associate the sample with the workflows and analysis that result from a sequencing run and analysis (eg, sample lineage and traceability). Enter the Universal Sample Identifier value during sample accessioning. If the identifier is not provided during sample accessioning, the Register Sample automation does not have access to this value at run time. The CLPA association between the samples and other analytics data is also restricted.

When you install any of the CLPA enabled workflows, the Illumina Universal Sample Identifier custom field is automatically created. If the CLPA enabled workflows are not installed, the custom field must be created manually. When using the Illumina Universal Sample Identifier, the recommended data format is `usi.<clarity_hostname>.<sampleId>`.

For more information on installing CLPA enabled workflows, contact Illumina Support.

Add the Illumina Universal Sample Identifier Submitted Sample custom field.

1. On the main menu, select Configuration, and then select the Custom Fields tab.
2. On the Global Fields tab, in the Submitted Sample list, select the plus icon to create a custom field.
3. Enter Illumina Universal Sample Identifier as the field name.
4. Set the following properties:
   a. For Field Type, select Text.
   b. For Required Field, select No.
   c. For Read Only, select No.
5. Select Save.

Use Common CLPA Automation

Common CLPA automation scripts are used for workflow customization. Each of these automation scripts logs a message into a log file that is defined with the parameter -l. Because steps can have different log placeholder positions, you must update the -l parameter to reference the correct log file placeholder (eg, `{compoundOutputFileLuid1}`).

The following automations can be used on any step.

- Step Started:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-
product-analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_
started.groovy'"
```

- Step Completed:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-
product-analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_
completed.groovy'"
```

The Register Sample automation can be used on the Library Prep step. If there are multiple steps in the Library Prep workflow, only add the Register Sample automation to the first step of the protocol. The script is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-
product-analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/common/register_
sample.groovy'
```

The Register Pool automation is available for the Pooling workflow. The script is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-
product-analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/pooling.groovy'
```

Workflow Customization

Customize the workflow with CLPA automation as follows.

1. Identify the type of CLPA automation to be added along with the trigger location and style.
   For more information on CLPA automation types and trigger location and styles, refer to *Automation Scripts* on page 2416.
2. Identify the protocol step to be customized.
3. Check for any existing automations on the triggers (eg, Step/Automatic upon entry or Step/Automatic upon exit). If there is an existing automation, modify the script as follows.
   a. Append the automation after the existing script with `&&`. Refer to the following examples.

   > When modifying an existing script, make sure to append the automation using the correct quotation marks ("). If you are appending an existing script on the next line, make sure that a backslash (\) is added on the last line of the script.

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- Before append:

```
bash -l -c "/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/<existingScript.jar>"
```

- After append:

```
bash -l -c "/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/<existingScript.jar> \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-
analytics/automation/unified-product-analytics-automation.jar \
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password}
\
-l {compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/common/step_
completed.groovy'"
```

b. Update the -l parameter to identify the correct Log File placeholder location. {compoundOutputFileLuid0} in the example refers to the first file placeholder.

c. If necessary, update the automation name to describe the new behavior.

d. In the Automation Use section, enable the step.

e. Save the automation.

4. If there is not an existing automation on the protocol step, check for an existing automation that has been configured as follows.

a. Identify the automation that is being used (eg, Register Step Started).

b. Make sure that the log file setting for the existing script has the -l parameter. This parameter identifies the same Log File placeholder that is used with the step.

- If the Log File location is the same, enable the step in the Automation Use section.
- If the Log File location is not the same, create a separate automation and enable the step in the Automation Use section of the new automation.

c. Save the automation.

5. Go to Configuration and select Lab Work.
6. Select the step and configure the Trigger Location/Style. Refer to *Automation Scripts* on page 2416. After the automation is enabled, the automation configuration option becomes available on the Step configuration page.
7. Save the step.

Clarity LIMS Product Analytics Integration v1.0 Configuration and User Interaction

The Clarity LIMS Product Analytics Integration v1.0 allows you to aggregate and analyze lab data from the workflow in a single location. The integration also integrates Clarity LIMS data into the Illumina Connected Analytics product analytics service. Data are uploaded to the ICA LINK service. A

subscription to ICA is required to enable the Clarity LIMS product analytics service.

Clarity LIMS Product Analytics Integration v1.0 is used with Clarity LIMS v6.0 and later. The integration supports on-site and hosted installations of Clarity LIMS. Illumina Preset Protocols v2.3 or later is required for automation.

Supported Workflows

Clarity LIMS Product Analytics Integration v1.0 supports the applicable workflows identified in *Supported Workflows* on page 2384.

Components

Clarity LIMS Product Analytics Integration v1.0 includes the following required components:

- BaseSpaceLIMS-unified-products-analytics—The core service of the Clarity LIMS Product Analytics Integration. This service installs the daemon service that publishes messages to the product analytics through the LINK service.
- BaseSpaceLIMS-unified-products-analytics-scripts—The automation scripts of the Clarity LIMS Product Analytics Integration. If new workflows or new product analytics scripts are introduced, this component can be upgraded.

For more information on the Clarity LIMS Product Analytics Integration scripts, refer to *Automation Scripts* on page 2426.

Terminology

As of Clarity LIMS v5, the term user-defined field (UDF) has been replaced with custom field in the user interface. However, the API resource is still called UDF.

There are two types of custom fields:

- Master step fields—Configured on master steps. Master step fields only apply to the following:
  - The master step on which the fields are configured.
  - The steps derived from those master steps.
- Global fields—Configured on entities (eg, submitted sample, derived sample, measurement, etc.). Global fields apply to the entire Clarity LIMS system.

Automation Scripts

The Clarity LIMS Product Analytics Integration v1.0 automation scripts are installed through the `BaseSpaceLIMS-unified-products-analytics-scripts` RPM. These scripts process Clarity LIMS data and prepare data for upload to the LINK service. There are two types of CLPA scripts: common and workflow-specific.

Common CLPA Scripts

Common CLPA scripts can be used with any compatible workflows. The following table lists and describes the common CLPA scripts.

Automation Name	Script Name	Trigger Location/Style	Purpose	Notes
Register Step Started	`common/step_started.groovy`	Location—Step Style—Automatic upon entry	Saves information on the step start time.	Can be used on any step.
Register Step Completed	`common/step_completed.groovy`	Location—Step Style—Automatic upon exit	Saves information on the step-completed time.	Can be used on any step.
Register Sample	`common/register_sample.groovy`	Location—Step Style—Automatic upon entry	Saves sample information used by the lab workflow.	Can be used on any step. It is recommended that this script is enabled at the first step of the workflow. Only one is required per workflow. This script requires the Submitted Sample field named Illumina Universal Sample Identifier.

For Research Use Only. Not for use in diagnostic procedures.

Automation Name	Script Name	Trigger Location/Style	Purpose	Notes
Register Pool	`common/pooling.groovy`	Location—Step Style—Automatic upon exit	Saves pool and library information used by the Clarity LIMS workflow.	Can be used on any pooling step. it is expected that libraries in the pool have been through the Add Labels step. Control samples do not need a label.

Workflow-Specific CLPA Scripts

Workflow-specific scripts work with Illumina workflows. These scripts require a specific set of custom fields and a specific protocol order in the workflow. The following table shows the workflow-specific scripts.

These scripts require custom fields to be available at their corresponding steps (listed under Clarity LIMS Product Analytics Automation Name and Remarks). Adding or enabling workflow-specific CLPA scripts on non-Illumina workflows is not supported. Data loss can occur.

As many of the scripts in this table share trigger location and style, they are combined into a single automation.

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Automation Name	Script Name	Used In	Trigger Location/Style	Purpose/Notes
Register NextSeq1k2k Run Association	`nextseq1k2k/nextseq1k2k_associate_seqrun.groovy`	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.2)	Location—Step Style—Automatic upon entry	Associates the sequencing run with the samples. This association links the Instrument Run ID with the sample IDs.
Register NextSeq1k2k RunStarted	`nextseq1k2k/nextseq1k2k_seqrun_started.groovy`	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.2)	Location—Step Style—Automatic upon entry	Records the started time of the sequencing run.

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Automation Name	Script Name	Used In	Trigger Location/Style	Purpose/Notes
Register NextSeq1k2k RunCompleted	`nextseq1k2k/nextseq1k2k_seqrun_completed.groovy`	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.2)	Location—Step Style—Automatic upon exit	Records the following information: • Run completion time • Run status • Run metrics This script requires the Cloud Run ID custom field from the Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.2) step.
Register NextSeq1k2k Run	`nextseq1k2k/register_nextseq1k2k_run.groovy`	AUTOMATED - Sequencing Run (NextSeq 1000/2000 Sequencing v2.2)	Location—Step Style—Automatic upon entry	Records the sequencing run configuration. This script requires all custom fields related to the run configuration in the Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.2) step.

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Automation Name	Script Name	Used In	Trigger Location/Style	Purpose/Notes
Register Analysis Completed	`nextseq1k2k/nextseq1k2k_analysisrun_completed.groovy`	Demultiplexing (NextSeq 1000/2000 Sequencing v2.2)	Location—Step Style—Automatic upon exit	Records the NextSeq1k2k analysis status and result. This script requires the Cloud Analysis Workflow Versions and Local Analysis Workflow Versions custom fields from the Load to Reagent Cartridge (NextSeq 1000/2000 Sequencing v2.2) step.
Register NovaSeq Run Association	`novaseq6000/novaseq_associate_seqrun.groovy`	AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.4) AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.6)	Location—Step Style—Automatic upon entry	Associates the sequencing run with the samples. This association links the Instrument Run ID with the sample IDs.

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Automation Name	Script Name	Used In	Trigger Location/Style	Purpose/Notes
Register NovaSeq RunStarted	`novaseq6000/novaseq_seqrun_started.groovy`	AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.4) AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.6)	Location—Step Style—Automatic upon entry	Records the started time of the sequencing run.
Register NovaSeq RunCompleted	`novaseq6000/novaseq_seqrun_completed.groovy`	AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.4) AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.6)	Location—Step Style—Automatic upon exit	Records the following information: • Run completion time • Run status • Run metrics

For Research Use Only. Not for use in diagnostic procedures.

Automation Name	Script Name	Used In	Trigger Location/Style	Purpose/Notes
Register NovaSeq Run	`novaseq6000/register_novaseq_run.groovy`	AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.4) AUTOMATED - NovaSeq Run (NovaSeq 6000 v3.6)	Location—Step Style—Automatic upon entry	Records the sequencing run configuration. This script requires all custom fields related to the run configuration. For Standard workflow: • Dilute and Denature (NovaSeq 6000 v3.4) • Dilute and Denature (NovaSeq 6000 v3.6) For XP workflow: • Load to Flow Cell (NovaSeq 6000 v3.4) • Load to Flow Cell (NovaSeq 6000 v3.6)

Automation Customization

Before customizing the automations in CLPA Integration v1.0, make sure that the following items are installed and configured:

- `BaseSpaceLIMS-unified-product-analytics`
- `BaseSpaceLIMS-unified-product-analytics-scripts`
- Illumina Preset Protocols (IPP) v2.3 or later (with at least one supported workflow)

Universal Sample Identifier

The Universal Sample Identifier is a Submitted Sample custom field used by the Register Sample automation. The field assigns a unique identifier to a sample across Illumina workflows. This identifier is used by CLPA to associate the sample with the workflows and analysis that result from a sequencing run and analysis (eg, sample lineage and traceability). Enter the Universal Sample Identifier value during sample accessioning. If the identifier is not provided during sample accessioning, the Register Sample automation does not have access to this value at run time. The CLPA association between the samples and other analytics data is also restricted.

When you install any of the CLPA enabled workflows, the Illumina Universal Sample Identifier custom field is automatically created. If the CLPA enabled workflows are not installed, the custom field must be created manually. When using the Illumina Universal Sample Identifier, the recommended data format is as follows.

`usi.<clarity_hostname>.<sampleId>.`

For more information on installing CLPA enabled workflows, refer to *Clarity LIMS Product Analytics Service Integration v1.0 Installation*.

Add the Illumina Universal Sample Identifier Submitted Sample custom field.

1. On the main menu, select Configuration, and then select the Custom Fields tab.
2. On the Global Fields tab, in the Submitted Sample list, select the plus icon to create a custom field.
3. Enter Illumina Universal Sample Identifier as the field name.
4. Set the following properties:
   a. For Field Type, select Text.
   b. For Required Field, select No.
   c. For Read Only, select No.
5. Select Save.

Use Common CLPA Automation

Common CLPA automation scripts are used for workflow customization. Each of these automation scripts logs a message into a log file that is defined with the parameter -l. Because steps can have different log placeholder positions, you must update the -l parameter to reference the correct log file placeholder (eg, `{compoundOutputFileLuid1}`).

The following automations can be used on any step.

- Step Started:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_started.groovy'"
```


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- Step Completed:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-
product-analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/step_
completed.groovy'"
```

The Register Sample automation can be used on the Library Prep step. If there are multiple steps in the Library Prep workflow, only add the Register Sample automation to the first step of the protocol. The script is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-
product-analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/common/register_
sample.groovy'
```

The Register Pool automation is available for the Pooling workflow. The script is as follows.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-
product-analytics/automation/unified-product-analytics-automation.jar
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} -s 'com/illumina/upa/scripts/common/pooling.groovy'
```

Workflow Customization

Customize the workflow with CLPA automation as follows.

1. Identify the type of CLPA automation to be added along with the trigger location and style.
   For more information on CLPA automation types and trigger location and styles, refer to *Automation Scripts on page 2426*.
2. Identify the protocol step to be customized.
3. Check for any existing automations on the triggers (eg, Step/Automatic upon entry or Step/Automatic upon exit). If there is an existing automation, modify the script as follows.
   a. Append the automation after the existing script with `&&`. Refer to the following examples.

   > When modifying an existing script, make sure to append the automation using the correct quotation marks (`"`). If you are appending an existing script on the next line, make sure that a backslash (`\`) is added on the last line of the script.

   - Before append:

   ```
   bash -l -c "/opt/gls/clarity/bin/java -jar
   /opt/gls/clarity/extensions/<existingScript.jar>"
   ```

   - After append:

   ```
   bash -l -c "/opt/gls/clarity/bin/java -jar
   /opt/gls/clarity/extensions/<existingScript.jar> \
   ```

```
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product-analytics/automation/unified-product-analytics-automation.jar \
script:executeUPAAutomationScript -i {stepURI:v2} -u {username} -p {password} \
-l {compoundOutputFileLuid0} -s 'com/illumina/upa/scripts/common/step_completed.groovy'"
```

   b. Update the -l parameter to identify the correct Log File placeholder location. {compoundOutputFileLuid0} in the example refers to the first file placeholder.
   c. If necessary, update the automation name to describe the new behavior.
   d. In the Automation Use section, enable the step.
   e. Save the automation.

4. If there is not an existing automation on the protocol step, check for an existing automation that has been configured as follows.
   a. Identify the automation that is being used (eg, Register Step Started).
   b. Make sure that the log file setting for the existing script has the -l parameter. This parameter identifies the same Log File placeholder that is used with the step.
      - If the Log File location is the same, enable the step in the Automation Use section.
      - If the Log File location is not the same, create a separate automation and enable the step in the Automation Use section of the new automation.
   c. Save the automation.
5. Go to Configuration and select Lab Work.
6. Select the step and configure the Trigger Location/Style. Refer to *Automation Scripts on page 2426*.
   After the automation is enabled, the automation configuration option becomes available on the Step configuration page.
7. Save the step.

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Lab Logic Tool Kit

Lab Logic Toolkit (LLTK) provides the evaluateDynamicExpression script, which allows for the evaluation of simple dynamic expressions—Excel-like formulas that can be used to perform calculations. The expressions may include full Java or Groovy syntax.

Each Clarity LIMS step comprises a series of mappings that relate a single input to a single output. The script applies and evaluates the expression for each input-output-mapping in the step.

For example, the following expression sets the next step for each output sample, depending on whether the input sample has passed or failed QC:

```
-exp 'if (input.QC == true) { nextStep = ::ADVANCE:: } else { nextStep =
::ESCALATE:: }'
```

Often, the formulas are used to set the values of one or more custom fields. However, LLTK can do a great deal more than simply manipulate field values.

Working with the Script

Script Parameters and Usage

The following table defines the parameters used by the evaluateDynamicExpression script.

Parameter	Description
-u {username}	Clarity LIMS login username. This parameter is required.
-p {password}	Clarity LIMS login password. This parameter is required.
-i {URI}	Clarity LIMS step URI. Provides context to resolve all token values. This parameter is required.
-t {true/false}	Test mode. The script may be executed automatically on step transition, or manually—by selecting the button on the Record Details screen. • For automatic script execution—set test mode to false. • For manual script execution—set test mode to true. Defaults to false if not specified. This parameter is optional.

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Parameter	Description
-h {true/false}	Halt on any error. By default, if the expression fails to evaluate, or evaluates to a non-numeric/non-sensical value, the script logs the problem and continue. A warning message advises the user to review the generated log file. Supplying the optional -h parameter with a value of true will cause the script to fail, preventing a step transition from proceeding, if any nonfatal errors arise. This parameter is optional.
-exp {expression}	The expression to be evaluated. The expression must be enclosed in single quotes, eg, -exp 'expression'. Refer to Using special characters - rules and constraints. This parameter is required.
-log {logFileLIMSID} -logFileName {logFileLIMSID}	The LIMS ID of the log file placeholder. This parameter is required.
-excludeControls {true/false} (Cannot be used withonlyControls; these two parameters are mutually exclusive)	Control sample handling. Set this parameter to true to make sure that: • The nextStep <next step value> (if used) is set to REMOVE for control samples. • Controls are excluded from any calculations in the expression. Refer to Setting next actions and Handling control samples. This parameter is optional.
-onlyControls {true/false} -onlyControlSamples {true/false} (Cannot be used withexcludeControls; these two parameters are mutually exclusive)	Control sample handling. Set this parameter to true to make sure that calculations are performed on control samples only. This parameter is optional.
-s {true/false} -stopwatch {true/false}	Tool logging. Set this parameter to true to record the time needed to run the tool as an entry in the log file. This parameter is optional.

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Automation Command Line

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar \-i {stepURI:v2:http} \-u {username} \-p {password} \script:evaluateDynamicExpression \-t <false/true> \-h <false/true> \-exp 'INSERT EXPRESSION HERE' \-log {logFileLIMSID}"
```

Using Special Characters—Rules and Constraints

Character Name	Character	Description
Single quote	'	As the 'exp' argument supplied to the script must be enclosed in single quotes, the single quote character cannot be used anywhere in the expression—including as part of any custom field that contains the single quote in its name.
Double quote	"	The entire command line must be enclosed in double quotes. Therefore, the double quote character cannot be used anywhere in the expression—including as part of any custom field that contains the double quote in its name.
Double colon	::	This two character string is used to delimit strings and custom field names. This character may not appear inside a custom field or string.

Joining Multiple Evaluations

Use the following special syntax to join multiple evaluations within an expression:

- AND = &&
- OR = ||
- NOT = !

Table 484 Examples

Expression	Description
A; B	Run A and then B, regardless of success of A.
A && B	Run B if A succeeded.
A || B	Run B if A failed.
if (!A) {B}	If A condition is not met, run B.

Boolean expressions should evaluate to true or false.

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Troubleshooting

For debugging errors, run through the following steps to determine the cause.

1. Check custom field format (for example, step.::UDF Name::).
2. Make sure that the custom fields have been created for the correct entity type.
3. Check the mapping of the field type (refer to section)—if doing Math they need to be numeric.
4. Check that the step has a log file placeholder configured. The 'unexpanded placeholders in string' error often refers to the last '-log' parameter—where the step has not been configured to produce one.
   If no log file is created, find additional error logging from the command line of the instance. This often shows the location of a syntax error. Find the log file at `/opt/gls/clarity/automation_worker/node/logs/automatedinformatics.log`.
5. Set -t parameter to false if an automatic trigger is used.

Data Collection Entities

When working with the Lab Logic Toolkit evaluateDynamicExpression script, custom field values can originate from the following data collection entities (these will differ, depending on the step from which the script is being run):

- input (analyte/derived sample)
- output (analyte/derived sample or per-input result file, measurement, or file placeholder*
- step
- container
- submittedSample / submittedSamples**

*Shared and per-reagent-label result files / measurements (eg, those produced by a demultiplexing step) are not supported.

**Use 'submittedSamples' with pooled derived samples, and 'submittedSample' for single-source derived samples.

Specifying Custom Fields

Each custom field is specified by using one of the data collection entity names (see *Data Collection Entities* on page 2440), followed by a dot separator, followed by the field name enclosed in :: characters.

For example:

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UDF/Custom Field Specification	Description
input.::Concentration (ng/mol)::	The value of the field Concentration (ng/mol) from the input sample
submittedSample.::Batch ID::	The value of the field Batch ID from the submitted sample
step.::Priority in Process::	The value of the field Priority In Process on the current step

Rules and Constraints

- The expression is evaluated and the target field value set for each input/output pair in the step.
- Where there are replicates or pooling this may mean that a particular input or output item is evaluated multiple times.
- Lab Logic Toolkit can write to read-only fields.
- Lab Logic Toolkit can write to all hidden fields.

The evaluateDynamicExpression script evaluates the expression and sets the target field value for each input/output pair in the step.

- Where there are replicates or pooling this may mean that a particular input or output item will be evaluated multiple times.
- The output data collection is treated specially. The script performs the following actions:
  - Searches the per-input output sample and the per-input output result file/measurement (if they exist) for any output field names found in the expression.
  - Returns any values found.
- In the event of a name collision (ie, the same field name exists on both sample output and result file / measurement output) the sample value is used.

Example Expressions

Example 1

```
-exp 'output.::DNA Concentration:: = (step.::Ideal Volume:: * step.::Ideal
Concentration::) / input.::Volume::
```

In this example expression, the sample output DNA Concentration field is assigned the value resulting from the following calculation:

1. Multiplying the value of the step Ideal Volume by the value of its Ideal Concentration.
2. Dividing that result by the Volume defined on the sample input.

Note the following:

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- step.::Ideal Volume:: is the value of a UDF/custom field. The period between step and Ideal Volume signifies that Ideal Volume is associated with the step.
- The part of the expression to the left of the = sign is always the destination or result of the expression, that is:

```
-exp 'destination = (step.::Ideal Volume:: * step.::Ideal Concentration::) /
input.::Volume::'
```

- The part of the expression to the right of the = sign is always the source of the value to be stored in the destination, that is:

```
-exp 'destination = source value'
```

- In this case, multiplying the value of the protocol step Ideal Volume by the value of its Ideal Concentration, and then dividing the result by the Volume defined on the sample input.

Example 2

Copy a step or analyte / derived sample field value to a field configured on the step output - for use in downstream steps:

```
-exp 'output.::PCR Cycles:: = step.::PCR Cycles::'
```

This expression will be evaluated one time for each output.

Example 3

Copy a result file or measurement field value to the output analyte / derived field for use in downstream step:

```
-exp 'output.::Concentration:: = input.::Concentration::'
```

Example 4

Automatically set a text-string field value:

```
-exp 'output.::Conc. Units:: = ::nM:: '
```

Handling Non-Existent UDFs/Custom Fields

In the API, fields that do not exist and fields that do not have a value set are considered the same and are handled in the same way.

Attempting to access a nonexistent UDF / custom field will usually result in an error. Sometimes this may not be the desired behavior.

Before attempting to access a field, you can use the hasValue function to check if it exists.

The hasValue function:

- May be used on any item.
- Takes one parameter - the name of the field.

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- Returns true if the field exists and has a non-null value.

Example Expressions:

The following expression checks that the Concentration field has a non-null value, and then checks that this value is greater than 1:

```
-exp 'input.::QC:: = input.hasValue( ::Concentration:: ) &&
input.::Concentration:: > 1'
```

The following expression checks that a toggle switch field has a non-null value - ie, that it is set to true or false, and then checks that the value is set to true:

```
-exp 'if (step.hasValue(::Toggle UDF::) && step.::Toggle UDF::)'
```

Mapping Field Types

The following tables summarize the mapping of UDF/custom field types.

Field Type	Groovy Data Type
Text	String
Multiline Text	String
Text Dropdown	String
Hyperlink	String
Hyperlink Dropdown	String
Toggle Switch	Boolean
Date	Date
Numeric	double, float, log, int, BigDecimal, BigInteger
Numeric Dropdown	double, float, log, int, BigDecimal, BigInteger

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Working with Toggle Switches

The LIMS and API treat the toggle switch field type as boolean when its value is set to true or false. However, it is possible to configure a toggle switch to not have to set a boolean true / false value. In this case, the value of the field is None Set and the field is treated as a 'nonexistent' field. To make sure that a toggle switch value has been set, you can use the hasValue function. For details, see *Handling Non-Existent UDFs/Custom Fields* on page 2442.

Setting QC Flags

In QC steps, some items have a QC flag associated with them. This flag can be read and set from script expressions. The value may be set to true or false.

Example expressions:

```
-exp 'input.QC = input.::Concentration:: >= step.::minConcentration:: &&
input.::Concentration:: <= step.::maxConcentration::' -exp 'output.QC =
input.QC'-exp 'if(Step.::Concentration:: >= 1) { input.QC = true } else {
input.QC = false }'
```

Setting Next Actions

The nextStep property is similar to the input, output, submittedSample, container, and step entities. However, nextStep can only be used on the left side of a dynamic expression; it cannot be used in the calculation on the right side of the expression.

The basic syntax is as follows:

```
nextStep = <next step value>
```

Depending on how the step is configured, a nextStep expression may set the next action for the step inputs or the step outputs. For example, in steps that do not produce output samples—such as QC steps, it is the step inputs that proceed (provided they pass QC) to the next step in the workflow.

When creating an expression that calls the nextStep property, there is no need to differentiate between step inputs and step outputs. The LIMS is able to determine this from how the API represents the entities that are available to set next actions on.

Note also that this property is evaluated for each input/output, and can set a different nextStep value for each.

Supported Values for nextStep

For samples to advance to the next step via an LLTK expression, the next step must be specified in the Configuration area of the LIMS.

Configure next steps on the protocol configuration form, in the Next Steps table.

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The possible next step values listed in the following table are intended for non-QC protocols. There is no nextStep entity for a QC protocol. However, samples in a QC step in a QC protocol can move to REMOVE or ESCALATE via LLTK.

<next step value> can be one of the following:

- Any Groovy expression that includes the top-level entity UDFs/custom fields on input, output, submitted Sample, and evaluates to one of the options described.
- A valid next step name, for example, : :Sample Pooling:: (remember that text / string values must be inside double colon delimiters).
- One of the following 'special values' described in the following table.

Table 485 nextStep Special Values

Special Value	Description
::ADVANCE::	Used to move the samples to the logical next step in the protocol (provided only one next step is permitted)
::REPEAT::	Used to select Repeat this step.
::REMOVE::	Used to remove sample from workflow.
::ESCALATE:	Used to select Request Manager Review. Reviewer name is left unset.
::COMPLETE::	Used to select Mark protocol as Complete.
::COMPLETE_REPEAT::	Used to select Complete and Repeat this step.
::COMPLETE_ REPEAT:<step name>::	Used to select Complete and Repeat this step, selecting the named step as the next step to continue with.
::REWORK:<step name>::	Used to select Rework to a previous step, selecting the named step as the step to rework the samples from.

The following conditions must be met:

- If <next step value> is not a valid next step, or is not one of the special values described in the table above, an error is raised and the script will fail by returning a nonzero value.
- The <next step value> must be in 'choose next steps' or 'record details' transition when the script is executed. If not, the script will fail and will return an error message.

Example expressions

```
-exp 'nextStep = ::ADVANCE::'
```

```
-exp 'if (input.QC == true) { nextStep = ::ADVANCE:: } else { nextStep =
::ESCALATE:: }'
```


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```
-exp 'if (output.QC == true) { nextStep = ::ADVANCE:: } else { nextStep =
::ESCALATE:: }'

-exp 'if (input.::QC:: == true) { nextStep = ::TP53 Make Master Mix Deep Well
Plate:: } else { nextStep = ::Dilute/Concentrate:: }'

-exp 'if (step.::Concentration:: >= 25) { input.QC = true } else {input.QC =
false }'
```

```
-exp 'if ( step.::Visual QC Result:: == ::Passed Visual QC:: ) { nextStep =
::SEMI-AUTOMATED - Make DNA Quant and Eval Standard Quant:: } else { nextStep =
::REMOVE:: }'

-exp 'if (input.::Avg Concentration:: > 25) { nextStep = ::Cluster Generation
(LLT):: } else { nextStep = ::ESCALATE:: }'
```

Handling Control Samples

When working with control samples, single-use control samples cannot have their next steps set to anything other than REMOVE.

Supplying the optional -excludeControls parameter with a value of true ensures the following:

- The script calculation is only evaluated for noncontrol samples.
- If next steps are set, the value is set to REMOVE for control samples.

Note also that the Java contains() method provides a simple way of identifying controls to be removed:

```
-exp 'if (input.name.contains( ::No Template Control::)) { nextStep = ::REMOVE::
}' -exp 'if (input.name.contains( ::CTLl::)) { nextStep = ::REMOVE:: }'
```

Working with Submitted Samples

For a pooled derived sample consisting of several submitted samples, Lab Logic Toolkit exposes a submittedSamples property that is a collection of the submitted samples represented in the well.

- submittedSamples returns a list of submitted samples.
- If its not a pooled sample it will return a list of 1.
- submittedSample returns an error if sample is pooled.

Use this collection to read and/or update all related submitted samples.

For example:

- To set the sample level UDF/custom field for each of the related submitted samples:

```
-exp 'submittedSamples.each { sample -> sample.::My Sample-Level UDF:: = 123 }'
```

- To sum the Volume UDF/custom field on all the related submitted samples into a single value written to the input analyte / derived sample:

```
-exp 'input.::Total Origin Sample Volume:: = submittedSamples.sum { sample ->
sample.::Volume:: }'
```

For information on Groovy syntax and closures, see Groovy Closures.

Working with Containers

The container entity makes the input, output, or submittedSample container available to the scripting engine.

- The container can be queried for its name, for example:

```
input.::container::.::name::
```

- Container name, UDFs / fields, and entity information can be read or written, for example:

```
output.::container::.::example udf1:: = input.::container::.::example udf1::
```

Example basic expressions:

```
-exp 'output.container.::containerUDF:: = ::This is a string::'-exp
'input.::container::.::containerUDF:: = ::This is a string::'-exp
'submittedSample.container.::containerUDF:: = ::This is a string::'
```

The word 'container' may be optionally surrounded by "::"

Non-UDF Custom Field Properties

The following table summarizes the non-UDF/custom field properties available for each entity type. Column headers are the types available. The bold row entries are the properties available.

Property	input sample	output sample	per input result file/measurement	submitted sample	container	step
name	yes	yes	yes	yes	yes	yes
container	yes	yes	yes	yes	no	no
workflowName	yes	yes	no	no	no	no
replicateCount	yes	no	no	no	no	no
pooledInputsCount	yes	yes	yes	no	no	no
well	yes	yes	yes	yes	no	no
LIMSID	Supported via Groovy node access. See limsid section.					

The name read-only property makes the name of the entity available to the scripting engine.

Example basic expression:

```
-exp 'input.::Latest Step:: = step.name'
```

workflowName

The workflowName read-only property makes the workflow name of input samples available to the scripting engine.

This property contains a string representing the current IN_PROGRESS workflow names. The string is formatted as a comma-delimited list, with a space inserted before each new item.

Example basic expressions:

```
-exp 'submittedSample.::Current Workflow:: = input.workflowName'-exp 'if
(input.workflowName.contains(::TruSeq Nano DNA Sample Prep::)) {
submittedSample.::Status:: = ::Prep:: }'-exp 'output.::Active Workflow Count:: =
input.workflowName.split(::, ::).size()'
```

Workflows are not assigned to output samples until the step that creates those samples completes (this will always be the current step). For this reason, the workflow name should always be retrieved from the input entity.

replicateCount

The replicateCount read-only property is available for input samples.

This property contains an integer value representing the number of output replicates (samples or result files / measurements or files / file placeholders generated from the input sample in the current step.

Example basic expressions:

```
-exp 'input.::Replicate Count UDF:: = input.replicateCount'-exp 'step.::Total
Replicates:: = step.::Total Replicates:: + input.replicateCount'
```

Because the replicateCount property is only available for input samples, its value for output samples will always be zero.

pooledInputsCount

The number of input analytes/derived samples that contributed to this analyte / derived sample.

- For pooled analytes / derived samples, this is > 1.
- For non-pooled analytes this is 1.
- Replicated samples that are then pooled are still counted separately in pooledInputsCount.

Example basic expression:

```
-exp 'output.::Count:: = input.pooledInputsCount'
```

When working with the pooledInputsCount property, the script looks at the step that created the derived sample or result file / measurement, and doesnot look upstream for a pooling step.

Example scenario:

Step 1: Pooling step that creates pooled samples.

Step 2: Nonpooling step that takes in the pooled samples and creates new derived samples (that are themselves still pools).

Step 3: Nonpooling step with expression that calls input.pooledInputsCount.

The expression in step 3 returns '1' because it onlys look at the previous step (step 2)—the step that created the current derived samples.

limsid

You can access the limsid field with some knowledge of Groovy and the LLTK. Use the following examples with any supported entity:

Example basic expressions:

```
input.node.@limsid
step.node.@limsid
output.container.node.@limsid
```

well

The well read-only property makes the well location of input, output, or submittedSample available to dynamic expressions. Result file / measurement outputs are supported if they have placement information (eg, on QC steps).

Example basic expression:

```
-exp 'output.::Input well:: = input.well'
```

Failing a Script

Use the fail() method to halt the script and display an error message. If the script is used on a step transition, this method also prevents the step from proceeding to the next screen.

Example expression:

```
-exp 'if (!step.hasValue( ::Batch ID:: )) { fail(::Batch ID step UDF is
required::) }'
```

Note that this example also uses the hasValue function (see *Handling Non-Existent UDFs/Custom Fields* on page 2442).

Working with Submitted Samples

For a pooled derived sample consisting of several submitted samples, Lab Logic Toolkit exposes a submittedSamples property that is a collection of the submitted samples represented in the well.

- submittedSamples returns a list of submitted samples.
- If its not a pooled sample it will return a list of 1.
- submittedSample returns an error if sample is pooled.

Use this collection to read and/or update all related submitted samples.

For example:

- To set the sample level UDF/custom field for each of the related submitted samples:

```
-exp 'submittedSamples.each { sample -> sample.::My Sample-Level UDF:: = 123 }'
```

- To sum the Volume UDF/custom field on all the related submitted samples into a single value written to the input analyte / derived sample:

```
-exp 'input.::Total Origin Sample Volume:: = submittedSamples.sum { sample ->
sample.::Volume:: }'
```

For information on Groovy syntax and closures, see Groovy Closures.

Comparing Stop/Start Dates and Times with LLTK

Lab scientists need to record both a start and stop date/time for an incubation. As a second evaluation of how many hours the incubation lasted, the scientists want BaseSpace Clarity LIMS to calculate the elapsed time.

The Lab Logic Toolkit evaluateDynamicExpression script can compare date/time entries and calculate elapsed hours. As Date fields in the LIMS do not display the required time component for this calculation, Text fields are needed.

The bash command uses a 'Date' function to convert the Text date/time entries into dates, and also uses a 'getTime' method to convert those dates into milliseconds since 1 January 1970 00:00:00 UTC. The difference between the two converted entries is then changed from milliseconds to hours.

Example

1. Create a step level Text field for the Start Date/Time. In this example, the field is named Incubation Start yyyy-mm-dd hh24:mi.
   The format is included in the name to assist the scientist entering the date/time.
2. Create a step level Text field for the Stop Date/Time.
   In this example, the field is named Incubation Stop yyyy-mm-dd hh24:mi.
3. Create a step level Numeric field for the Incubation Hours. Set your preferred decimal place limit.
4. Create an automation and enter the following command line (if you have used different field names, edit the example command line to match):

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
script:evaluateDynamicExpression -exp 'step.::Incubation Hours:: = (Date.parse
(::yyyy-mm-dd H:m::, step.::Incubation Stop yyyy-mm-dd hh24:mi::).getTime() -
Date.parse(::yyyy-mm-dd H:m::, step.::Incubation Start yyyy-mm-dd
hh24:mi::).getTime()) / (1000*60*60)' -log {compoundOutputFileLuid0}"
```

5. Set the automation to be triggered by a button on the step. This allows the scientist to enter/edit the Start and Stop Date/Time before the elapsed hours are calculated.

Adjustment for the Start/Stopevents taking place over the 'daylight savings change events' is not included in this example.

If you want to use a button to populate a Text field with the current date/time, then use:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -exp 'step.::Formulation:: = new Date().getDateTimeString()' -log {compoundOutputFileLuid0}"
```

Lab Logic Toolkit FAQ

What Java/Groovy methods can I use in a Lab Logic Toolkit (LLTK) Expression?

You can use all methods included in the Java and Groovy base packages.

Commonly used Java classes are:

- String: http://docs.oracle.com/javase/7/docs/api/java/lang/String.html
- Math: https://docs.oracle.com/javase/7/docs/api/java/lang/Math.html

For information on Groovy closures, see Groovy Closures.

How do I handle attempts to access custom fields that do not exist or do not have a value set?

Use the hasValue function to check for nonexistent fields and fields that do not have a value set - including toggle switches in LIMS v5 and later.

See *Non-UDF Custom Field Properties* on page 2447.

How do I prevent a user from moving to the next screen if certain criteria are not met?

Use the hasValue function together with the fail() method to check for step UDF / custom field values when the Record Details screen is exited, and prevent the user from advancing to the next screen if they have not entered values for these fields.

See *Failing a Script* on page 2449.

How do I control the number of decimal places for a custom field?

Use the following expression:

1. Use Groovy syntax to format the following string:

```
String.format(::%.0f::, step.::counter::)
```

2. Replace step.::counter:: with the field you want to apply the formatting to.

3. Replace %.0f with the number of decimal places needed. For example, for three decimal places, use %.3f.
   Example:

```
String.format(::%.2f::, Concentration::).
```

How do I round to the closest integer below a value using Math.floor?

Use the following expression:

```
-exp 'output.::NTP Uses:: = Math.floor((input.::NTP Volume (uL):: - step.::NTP Dead Volume (uL)::)/ step.::NTP Volume Per Lane (uL)::)'
```

How do I use LLTK with required custom fields?

Custom fields are set as a bulk group in the API. The API checks all required fields.

When LLTK updates a field, all required fields must be set or an error will occur.

Workaround:

1. Set the required fields with a value such "Please fill in this value".
2. Use another LLTK expression triggered on step completion to check whether this value has been updated.
   - If the field value has been updated, allow the step to proceed.
   - If the field value has not been updated, log an error and halt progress of the step.

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Lab Instrument Toolkit

Lab Instrument Toolkit provides a number of scripts that can be used for custom configuration.

- The driver_file_generator script (Template File Generator) is a file-generation solution that produces custom template files without requiring scripting or development knowledge or resources.
- The addBlankLines script allows for the creation of files that require a line entry for every well in a container, including those wells that are empty.
- The convertToExcel script converts separated-value files (eg. CSV) to Microsoft Excel XLS or XLSX spreadsheet format.
- The parseCSV script allows for the data for each well to be parsed into fields on either derived samples or measurement records that map directly to the derived samples being measured.
- The parseXmlBySampleName script matches data in a result file to samples in the LIMS, using the measurement record LIMSID.
- The PlacementHelper script automates sample placement according to a transfer file produced by a robot. The script covers a one-to-one, many-to-one (pooling), or one-to-many (replicates) mapping of samples for placement.

Template File Generator

The Template File Generator is a file-generation solution that allowsClarity LIMS admins, such as lab managers, to produce custom template files without requiring scripting or development knowledge or resources.

At run time, the Template File Generator uses a script (driver_file_generator) and the supplied template file to generate a file. This may be a simple file that includes a subset of LIMS data, or a more complex sample sheet file for upload to the sequencing instrument to start a run.

The format of a template file is typically a comma-delimited CSV file. However, the following file formats are also supported: .bak, .groovy, .md5, .tsv, .txt, .xml.

How the Template File Generator Works

1. In Clarity LIMS:
   - An automation is configured and enabled on a step.
   - The driver_file_generator script is triggered from the automation command line.
2. The script uses the template file to generate a file, the contents of which are based on the specifications provided in the template.
3. The script extracts data from the LIMS via the API, based on tokens defined within the template file.

4. The script parses the template file and processes the sections, metadata, and tokens it contains. Sections may include header block, header, data, and footer, each of which is enclosed inside tags.

For example:

```
<HEADER_BLOCK> Header block entry one, two, three More information</HEADER_
BLOCK><DATA> Data row entry Data row entry</DATA>
```

For details on template sections and the tokens you can use in your template files, see.

Several special options are available for inclusion in the template. These options do not directly pull data from the LIMS API. Instead, they modify what has been gathered in the template file.

For details, refer to *TOKEN FORMAT* on page 2460.

Creating Template Files

You can create template files that the Template File Generator script (driver_file_generator) will use to generate custom files for use in your lab.

This article provides details on the following:

- The parameters used by the script.
- The sections of the template file—these define what is output in the generated file.
- Sorting logic—options for sorting the data in the generated file.
- Rules and constraints to keep in mind when creating templates and generating files.
- Examples of how you can use specific tokens and metadata in your template files.

For a complete list of the metadata elements and tokens that you can include in a template file, see *Template File Contents* on page 2473.

Script Parameters

Upgrade Note: process vs step URIs

The driver_file_generator script now uses steps instead of processes for fetching information. When a process URI is supplied, the script detects it and automatically switches it to a step URI. (The PROCESS.TECHNICIAN token, which is only available on 'process' in the API, is still supported.)

The behavior of the script has not changed, except that the long form -processURI parameter must be replaced by -stepURI in configuration. The -i version of this parameter remains supported and now accepts both process and step URI values.

If your configuration is using -processURI or --processURI, replace each instance with -i (or -stepURI/--stepURI).

The following table defines the parameters used by the driver_file_generator script. For details on the metadata and tokens referenced in the table, see *Template File Contents* on page 2473*Metadata* on page 2473

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Table 486 Script Parameters

Option	Name	Description
-i {stepURI:v2} -stepURI {stepURI:v2}	Step URI	(Required) LIMS step URI Provides context to resolve all token values. See Upgrade note.
-u {username} -username {username}	Username	(Required) LIMS login username
-p {password} -password {password}	Password	(Required) LIMS login password
-t <templateFile> -templatePath <templateFile>	Template file	(Required) Template file path
-o <outputFile> -outputPath <outputFile>	Output file	(Required) Output file path If the folder structure specified in the path does not exist, it is created. • This output file parameter value is overwritten by OUTPUT.FILE.NAME • To output multiple files, use GROUP.FILES.BY.INPUT.CONTAINERS and GROUP.FILES.BY.OUTPUT.CONTAINERS • Files generated are in CSV format by default. Other value-separated formats are available—see OUTPUT.SEPARATOR.
-l <logFile> -logFileName <logFile>	Log file	(Required) Log file name

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Option	Name	Description
-q \[true|false] -quickAttach \ [true|false]	Quick Attach	Default is 'false'. Provide as 'true' to attach the file on script completion. To attach manually or with AI/Automation Worker, name the file starting with the placeholder LIMSID. If multiple files are generated, they are zipped into one archive. Main use cases are: • Multiple files are generated (see GROUP.FILES.BY) and must be attached to the LIMS (in addition to, or in place of, writing them to disk). • When chaining multiple scripts together, this make sure that the file has already been attached before the next script runs. See *Renaming generated files* on page 2467 and *Generating Multiple Files* on page 2470 examples.
-destLIMSID <LIMSID>	Destination LIMS ID	LIMSID of the output to attach the template file to. Use with quickAttach. See *Renaming generated files* on page 2467 and *Generating Multiple Files* on page 2470 examples.

Command-line example:

```
bash -l -c "opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/DriverFileGenerator.jar script:driver_file_generator -i {stepURI:v2} -u {username} -p {password} -t /opt/gls/clarity/customextensions/InfiniumHT/driverfiletemplates/NextSeq.csv-o {compoundOutputFileLuid0}.csv -l {compoundOutputFileLuid1}"
```

Command-line example using -quickAttach and -destLIMS:

```
bash -l -c "/opt/gls/clarity/bin/java -cp /opt/gls/clarity/extensions/ngs-common/v5/EPP/DriverFileGenerator.jar script:driver_file_generator -i {stepURI:v2} -u {username} -p {password} -t /opt/gls/clarity/customextensions/Robot.csv -quickAttach true -destLIMSID {compoundOutputFileLuid0} -o extended_driver_x384.csv -l {compoundOutputFileLuid2}"
```

See also the *Template File Examples* on page 2463 section.

Data Source

The input-output-maps of the step (defined by the -stepURI parameter) are used as the data source for the content of the generated file.

If they are present, input-output-maps with the attribute output-generation-type=PerInput are used. Otherwise, all input-output-map items are used.

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By default, the data source entries are sorted alphanumerically by LIMS ID. You can modify the sort order by using the SORT.BY and SORT.VERTICAL metadata elements.

The output generation type specifies how the step outputs were generated in relation to the inputs. PerInput entries are available for the following step types: Standard, Standard QC, Add Labels, and Analysis.

Template Sections

The content of the generated file is determined by the sections defined in the template. Content for each section is contained within xml-like opening and closing tags that are structured as follows:

```
<SECTION> section content</SECTION>
```

Most template files follow the same basic structure and include some or all the following sections (by convention, section names are written in capital letters, but this is not required):

<HEADER_BLOCK><HEADER><DATA><FOOTER>

The order of the section blocks in the template does not affect the output. In the output file, blocks will always be in the order shown.

The area outside of the sections can contain metadata elements. Anything else outside of the section tags is ignored.

The <PLACEMENT> and <TOKEN FORMAT> sections are not part of the list and do not create distinct sections in the generated file. Instead, they alter the formatting of the generated output.

HEADER_BLOCK

Only a subset of the tokens is available for use in the header block section. (For details, see *Tokens* on page 2481 table).

If an unsupported token is included, file generation will complete with a warning message and a warning will appear in the log file.

The header block section may include both plain text and data from the LIMS. It consists of information that does not appear multiple times in the generated file—ie, the information is not included in the data rows (see DATA section)

Tokens in the header block always resolve in the context of the first input and first output available. For example, suppose the INPUT.CONTAINER.TYPE token is used in the header block:

- If there is only one type of input container present in the data source, that container type will be present in the output file.
- If multiple input container types are present in the data source, only the first one encountered while processing the data will be present in the output file.

For this reason, we recommend against using tokens that will resolve to different values for different samples - such as SAMPLE.NAME. If one of these tokens is encountered, a warning is logged and the first value retrieved from the API is used. (Note that you may use.ALL tokens, where available.)

To include a header block section in a template, enclose it within the <HEADER_BLOCK> and </HEADER_BLOCK> tags.

HIDE feature: If one of the tokens of a line is empty and is part of a HIDE statement, that line will be removed entirely. See *Using HIDE to Exclude Empty Columns* on page 2470 and *Using HIDE to Exclude Empty HEADER rows* on page 2470examples.

HEADER

The header section describes the header line of the data section (see DATA section). A simple example might be "Sample ID, Placement".

The content of this section can only include plain text and is output as is. Tokens are not supported.

To include a header section in a template, enclose it within the <HEADER> and </HEADER> tags.

HIDE feature: See ' Hide feature' in DATA section. Also note:

- If multiple <HEADER> lines are present, at least one must have the same number of columns as the <DATA> template line.
- <HEADER> lines that do not match the number of columns are unaffected by the HIDE feature.

DATA

Each data source entry creates a data row for each template line in the section. All entries are output for the first template line, then the next template line runs, and so on.

The data section allows tokens and text entries. All tokens are supported.

Note the following:

- Duplicated rows are eliminated, if present. A row is considered duplicated if its content (after all variables and placeholders have been replaced with their corresponding values) is identical to a previous row. Tokens must therefore provide distinctive enough data (ie, something more than just CONTAINER.NAME) if all of the input-output entry pairs are desired in the generated file.
- By default, the script processes only sample entries. However, there are metadata options that allow inclusion of result files/measurements and exclusion of samples.
- Metadata sorting options are applied to this section of the template file only.
- By default, pooled artifacts are treated as a single input artifact. They can be demultiplexed using the PROCESS.POOLED.ARTIFACTS metadata element.
- If there is at least one token relevant to the step inputs or outputs, this section will produce a row for each PerInput entry in the step input-output-map. If no PerInput entries are present in the step input-output-map, the script will attempt to add data rows for PerAllInputs entries.

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- Input and output artifacts are always loaded if a <DATA> section is present in the template file, due to the need to determine what type of artifacts the script is dealing with.

To include a data section in a template, enclose it within the <DATA> and </DATA> tags.

HIDE feature: If the token in a given column is empty for all lines and that token is part of a HIDE statement, that column (including the matching <HEADER> columns) will be removed entirely. There can only be one <DATA> template line present when using the HIDE feature.

See *Using HIDE to Exclude Empty Columns* on page 2470 and *Using HIDE to Exclude Empty HEADER rows* on page 2470examples.

FOOTER

The content of this section can only include plain text and is output as is. Tokens are not supported.

To include a footer section in a template, enclose it within the <FOOTER> and </FOOTER> tags.

PLACEMENT

This section contains groovy code that controls the formatting of PLACEMENT tokens (see the PLACEMENT tokens in *Tokens* on page 2481 table).

Within the groovy code, the following variables are available:

Variable Name	Description
containerTypeNode	The container type holding the derived sample
row	The row part of the derived sample's location
column	The column part of the derived sample's location

Note the following:

- The script must return a string, which replaces the corresponding <PLACEMENT> tag in the template.
- Logic within the placement tags can be as complex as needed, provided it can be compiled by a groovy compiler.
- If an error occurs while running formatting code, the original location value is used.

To include a placement section in a template, enclose it within the <PLACEMENT> and </PLACEMENT> tags.

Placement Example: ContainerType

In the following example:

- If the container type is a 96 well plate, sample placement A1 will return as "A_1"

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- If the container type is not a 96 well plate, sample placement A1 will return as "A:1"

```
<PLACEMENT>
   // The inputs to this segment are: String row, String column, Node
   containerTypeNode
   if (containerTypeNode.@name == "96 well plate")
      return row + "_" + column
   else
      return row + ":" + column
</PLACEMENT>
```

Placement example: zero padding

```
<PLACEMENT>
   // The inputs to this segment are: String row, String column, Node
   containerTypeNode
   String zeroPad (String entry) {
      if (entry.isNumber() && entry.size() == 1) return "0" + entry
      return entry
   }
   return zeroPad(row) + ":" + zeroPad(column)
</PLACEMENT>
```

TOKEN FORMAT

This section defines logic to be applied to specific tokens to change the format in which they appear in the generated file.

Special formatting rules can be defined per token using the following groovy syntax:

```
${token.identifier}… groovy code… // or ${token.identifier##Name}… groovy code…
```

Within the groovy code, the variable 'token' refers to the original value being transformed by the formatting code. The logic replaces all instances of that token with the result.

${token.identifier} marks the beginning of the token formatting code and the end of the previous token formatting code (if any).

- You can define multiple formatting logic rules for a given token, by assigning a name to the formatting section (named formatters are called 'variations'). This is done by appending “##” after the token name (eg “${token.identifier##formatterName}”).
- Using the named formatter syntax without giving a name (“${token.identifier##}”) will abort the file generation.
- If an error occurs while running formatting code, the resulting value will be blank.
- If a named formatter is used but not defined, the value is used as is.

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To include a placement section in a template, enclose it within the <TOKEN_FORMAT> and </TOKEN_FORMAT> tags.

TOKEN FORMAT Example: Technician Name

In this example, a custom format is defined for displaying the name of the technician who ran a process (step).

The name of the token appears at the beginning of the groovy code that will then be applied. In this code, the variable 'token' refers to the token being affected. The return value is what will replace all instances of this token in the file.

```
<TOKEN_FORMAT>
   ${PROCESS.TECHNICIAN}
   def name = token.split(" ")
   return "First name: " + name[0] + ", Last name: " + name[1]
</TOKEN_FORMAT>
```

TOKEN FORMAT Example: Container Name and String

In this example, a string is appended to the end of the input container name.

```
<TOKEN_FORMAT>
   ${INPUT.CONTAINER.NAME}
   return token + "-PlateName"
</TOKEN_FORMAT>
```

Metadata

Metadata provides information about the template file that is not retrieved from the API—such as the file output directory to use, and how the data contents should be grouped and sorted.

Metadata is not strictly confined to a section, and is not designated by opening and closing tags. However, each metadata entry must be on a separate line.

Metadata entries can be anywhere in the template, but the recommended best practice is to group them either at the top or the bottom of the file.

For a list of supported metadata elements, rules for using them, and examples, see *Template File Contents* on page 2473, Metadata section.

Sorting Logic

Sorting in the generated file is done either alphanumerically or by vertical placement information, using the SORT.BY. and SORT.VERTICAL metadata elements.

Sorting must be done using a combination of sort keys - provided to SORT.BY. as one or more ${token} values, each of which always produces a unique value in the file. For example, sorting by just OUTPUT.CONTAINER.NAME would work for samples placed in tubes, but would not work for samples in 96 well plates. Sorting behavior on nonunique combinations is not guaranteed to be predictable.

To sort vertically:

Include the SORT.VERTICAL metadata element in the template file. In addition, the SORT.BY.${token}, ${token} metadata must also be included, as follows:

```
SORT.BY.${OUTPUT.CONTAINER.ROW}${OUTPUT.CONTAINER.COLUMN}
```

Any SORT.BY. tokens will be sorted using the vertical sorter instead of the alphanumeric sort.

To apply sorting to samples in 96 well plates:

You could narrow the sort key to a unique combination such as:
SORT.BY.${OUTPUT.CONTAINER.NAME}${OUTPUT.CONTAINER.ROW}${OUTPUT.CONTAINER.COLUMN}

See also SORT.VERTICAL and SORT.BY in *Template File Contents* on page 2473, Metadata section.

Rules and Constraints

The template must adhere to the following rules:

- Metadata entries must each appear on a new line and be the only entry on that line.
- Metadata entries must not appear inside tags.
- Opening and closing section tags must appear on a new line and as the only entry on that line.
- Each opened tag must be closed, otherwise it is skipped by the script.
- Any sections (opening tag + closing tag combination) can be omitted from the template file.
- Entries that are separated by commas in the template will be delimited by the metadata-specified separator (default: COMMA) in the template file.
- White space is allowed in the template. However, if there is a blank line inside a tag, it will also be present in the template file produced.
- If an entry in the template is enclosed in double quotes it will be imported as a single entry and written to the template file as such, even if it has commas inside.
- To include double-quotes or single-quotes in the template file, use the escape character: Example: \" or \'
- To include an escape character in the template file, use two escape characters inside double-quotes. For example, if you want to see \\Share\Folder\Filename.txt use "\\\\Share\\Folder\\Filename.txt" as the token.

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If any of the following conditions is not met - the tag, and everything inside it, is ignored by the script and a warning displays in the log file:

- Except for the metadata, all template sections must be enclosed inside tags.
- Each tag must have its own line, and must be the only tag present on that line.
- No other entries, even empty ones, are allowed.
- All opened tags must be closed.
- Custom field names must not contain periods.

Template File Examples

The following examples show how to use tokens and metadata elements in template files. For a list of all supported tokens and metadata elements, see *Template File Contents* on page 2473 .

Illumina Instrument Sample Sheets

The LIMS provides configuration to support generation of sample sheets that are compatible with some Illumina instruments. For details, see the Illumina Instrument Integrations documentation.

Generating Sample Sheets for QC Instruments

The LIMS provides configured automations that generate sample sheets compatible with some QC instruments.

Generate Bioanalyzer Driver File Automation

Command line:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -u {username} -p {password} \
    script:driver_file_generator \
    -i {processURI:v2} \
    -t /opt/gls/clarity/extensions/ngs-common/v5/EPP/conf/readonly/bioA_driver_
    file_template.csv \
    -o {compoundOutputFileLuid0}.csv \
    -l {compoundOutputFileLuid1} \
    && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
    common/v5/EPP/ngs-extensions.jar
    -u {username}
    -p {password} \ script:addBlankLines \
    -i {stepURI:v2} \ -f {compoundOutputFileLuid0}.csv \
    -l {compoundOutputFileLuid1} \
    -sep COMMA \
```

```
   -b ',False,' \
   -h 1 \
   -c LIMSID \
   -pre 'Sample '"
```

Template file content:

```
INCLUDE.OUTPUT.RESULTFILES
<HEADER_BLOCK>
</HEADER_BLOCK>
<HEADER>
   "\"Sample Name\",\"Sample Comment\",\"Rest. Digest\",\"Observation\""
</HEADER>
<DATA>
   ${OUTPUT.LIMSID},,False,
</DATA>
<FOOTER>
   Ladder,,False,"\"
   Chip Lot #\",\"Reagent Kit Lot #\"
   ,
   \"QC1 Min [%]\",\"QC1 Max [%]\",\"QC2 Min [%]\",\"QC2 Max [%]\"
   ,,,
   \"Chip Comment\”
   "
</FOOTER>
```

Generate NanoDrop Driver File Automation

Command line:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -u {username} -p {password} \
   script:driver_file_generator \
   -i {processURI:v2} \
   -t /opt/gls/clarity/extensions/ngs-common/v5/EPP/conf/readonly/nd_driver_
   file_template.csv \
   -o {compoundOutputFileLuid0}.csv \
   -l {compoundOutputFileLuid1}"
```

Template file content:

```
INCLUDE.OUTPUT.RESULTFILES
<HEADER_BLOCK>
</HEADER_BLOCK>
```

```
<HEADER>
   "Well Location, Sample Name"
</HEADER>
<DATA>
   ${INPUT.CONTAINER.PLACEMENT},${INPUT.CONTAINER.NAME}_
   ${INPUT.CONTAINER.PLACEMENT}_${INPUT.NAME}
</DATA>
```

Generate Tape station Input Sample table CSV Automation

Command line:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -u {username} -p {password} \
   script:driver_file_generator \
   -i {processURI:v2} \
   -t /opt/gls/clarity/extensions/ngs-common/v5/EPP/conf/readonly/tapestation_
   driver_file_template.csv \
   -o {compoundOutputFileLuid0}.csv \
   -l {compoundOutputFileLuid1}"
```

Template file content:

```
INCLUDE.OUTPUT.RESULTFILES
SORT.BY.${INPUT.LIMSID}
<DATA>
   ${OUTPUT.LIMSID}_${INPUT.NAME}
</DATA>
```

Create GenomeStudio® Driver File

Command line:

```
bash -c "/opt/gls/clarity/bin/java -cp /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar driver_file_generator \-i {processURI} -u
{username} -p {password} -t
/opt/gls/clarity/extensions/conf/driverfiletemplates/GenomeStudioGeneExpressionT
emplate.csv \-o {compoundOutputFileLuid0}.csv -l {compoundOutputFileLuid1}.html"
```

Template file content:

```
INCLUDE.OUTPUT.RESULTFILES
OUTPUT.SEPARATOR,COMMA
LIST.SEPARATOR,";"
ILLEGAL.CHARACTERS,COMMA
```

```
ILLEGAL.CHARACTER.REPLACEMENTS,_
SORT.BY.${INPUT.CONTAINER.NAME}${INPUT.CONTAINER.ROW}${INPUT.CONTAINER.COLUMN}
<HEADER_BLOCK>
   [HEADER]Investigator Name ${PROCESS.TECHNICIAN}
   Project Name, ${SAMPLE.PROJECT.NAME.ALL}
   Experiment Name
   Date, ${DATE}
   [Manifests]
   ${PROCESS.UDF.Manifest A}
   </HEADER_BLOCK>
<HEADER>
   [DATA]
   Sample_Name,Sample_Well,Sample_Plate,Pool_ID,Sentrix_ID,Sentrix_Position
</HEADER>
<DATA>
   ${INPUT.NAME},,,,${INPUT.CONTAINER.NAME},${INPUT.CONTAINER.PLACEMENT}
</DATA>
<PLACEMENT>
   // inputs to this section are String row, String column, Node
   containerTypeNode
   int convertAlphaToNumeric(String letters)
   {
      int result = 0letters = letters.toUpperCase()
      for (int i = 0; i < letters.length(); i++) {
         result += (letters.charAt(i).minus('A' as char) + 1) * (26 **
         (letters.length() - i - 1))
      }
      return result
   }
   int SENTRIX_POS_THRESHOLD = 12
   int WELL_PLATE_SIZE_96 = 96
   int xSize = containerTypeNode.'x-dimension'.size.text().toInteger()
   int ySize = containerTypeNode.'y-dimension'.size.text().toInteger()
   int containerSize = xSize * ySize
   boolean xIsAlpha = containerTypeNode.'x-dimension'.'is-alpha'.text
   ().toBoolean()
   boolean yIsAlpha = containerTypeNode.'y-dimension'.'is-alpha'.text
   ().toBoolean()
   if (containerSize <= SENTRIX_POS_THRESHOLD && (xIsAlpha || yIsAlpha)) {
      return row
```


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```
    }
    // R001_C001 for 96 well plate, r01c01 for other container types
    if (containerSize == WELL_PLATE_SIZE_96) {
        def numFormat = java.text.NumberFormat.getNumberInstance()
        numFormat.setMinimumIntegerDigits(3)
        String xStr = numFormat.format(column.isInteger() ? column as int :
        convertAlphaToNumeric(column))
        String yStr = numFormat.format(row.isInteger() ? row as int :
        convertAlphaToNumeric(row))
        // Row is mapped to x coordinate, while column is mapped to y.
        // When creating an array type of size 96, swap the row and column
        dimension.
        // e.g 12 x 8 array should be mapped as an 8 x 12 array
        //
        // This mapping has been in RI for a while.
        // In AddIlluminaArraysStep, all 2D Illumina arrays added have a dimension
        of 8 x 12.
        // This driver file template then converts it back to 12 x 8.
        // This logic is now corrected to follow other arrays to make sure the
        driver file.
        // generated is compatible with existing arrays and software.
        return "R"+xStr+"_C"+yStr
    } else {
        def numFormat = java.text.NumberFormat.getNumberInstance()
        numFormat.setMinimumIntegerDigits(2)
        String xStr = numFormat.format(column.isInteger() ? column as int :
        convertAlphaToNumeric(column))
        String yStr = numFormat.format(row.isInteger() ? row as int :
        convertAlphaToNumeric(row))
        // row is mapped to y, column is mapped to x
        return "r"+yStr +"c"+xStr
    }
</PLACEMENT>
```

Renaming generated files

In the template file, the following OUTPUT.FILE.NAME metadata element renames the generated template file 'NewTemplateFileName':

OUTPUT.FILE.NAME, NewTemplateFileName.csv

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In the automation command line, the following will attach the generated file to the {compoundOutputFileLuid0} placeholder, with the name defined by the OUTPUT.FILE.NAME metadata element.

```
bash -c "/opt/gls/clarity/bin/java -cp /opt/gls/clarity/extensions/ngs-common/v5/EPP/DriverFileGenerator.jar
    script:driver_file_generator
    -i {stepURI:v2}
    -u {username}
    -p {password}
    -t /opt/gls/clarity/customextensions/Robot.csv
    -q true -destLIMSID {compoundOutputFileLuid0}
    -o extended_driver_x384.csv
    -l {compoundOutputFileLuid2}
```

When the LIMS attaches a file to a placeholder in the LIMS, it assumes that the file is named with the step LIMSID, and uses this LIMSID to identify the placeholder to which the file should be attached.

However, when using OUTPUT.FILE.NAME, you can give the file a name that does not begin with the LIMSID of the placeholder to which it will be attached. To do this, you must use the quickAttach and destLIMSID parameters in the automation command line.

- If the quickAttach parameter is provided without destLIMSID parameter, the script logs an error and stops execution.
- If destLIMSID is provided without using quickAttach, it is ignored.

Using Token Values In File Names

The OUTPUT.FILE.NAME and OUTPUT.TARGET.DIR metadata elements support token values. This allows you to name files based on input / output values of the step - the input or output container name, for example.

The following tokens are supported for this feature:

- PROCESS.LIMSID
- PROCESS.UDF.<UDF NAME>
- PROCESS.TECHNICIAN
- DATE
- INPUT.CONTAINER.NAME
- INPUT.CONTAINER.TYPE
- INPUT.CONTAINER.LIMSID
- OUTPUT.CONTAINER.NAME

- OUTPUT.CONTAINER.TYPE
- OUTPUT.CONTAINER.LIMSID

Rules and Constraints

When using token values in file names, the following rules and constraints apply:

- Container-related functions will return the value from a single container, even if there are multiple containers.
- Other tokens will function, but will only return the value for the first row of the file (first input or output).
- If the OUTPUT.FILE.NAME specified does not match the LIMS ID of the file, the output file will not be attached in the LIMS user interface. To ensure that the file is attached, include the quickAttach and destLIMSID parameters in the command-line string.
- It is highly recommended that you do not use SAMPLE.PROJECT.NAME.ALL or SAMPLE.PROJECT.CONTACT.ALL, because the result is prone to surpassing the maximum length of a file name. There are similar issues with other SAMPLE tokens when dealing with pools.
- Only the following characters are supported in the file name. Any other characters will be replaced by an _ (underscore) by default. This replacement character can be configured with the OUTPUT.FILE.NAME.ILLEGAL.CHARACTER.REPLACEMENT metadata element.a-z.
  - A-Z
  - 0–9
  - _ (underscore)
  - - (dash)
  - . (period)

> Providing a full file path for OUTPUT.FILE.NAME is still supported, but deprecated. If the full path is provided, the file/directory separator will be automatically detected and will not be replaced in the static parts of the file name. Any of these separators derived from the result of a token value will be replaced.

Defining a Project Name for Control Samples

You can use the CONTROL.SAMPLE.DEFAULT.PROJECT.NAME metadata element to define a project name for control samples. The value specified by this token will be used when determining one or more values for the SAMPLE.PROJECT.NAME and SAMPLE.PROJECT.NAME.ALL tokens.

Example:

```
CONTROL.SAMPLE.DEFAULT.PROJECT.NAME, My Control Sample Project
```


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Rules and Constraints

- If the token is found in the template, but with no value then no project name will be given for control samples.
- If the token is not found in the template, then no project name will be given for control samples.
- If multiple values are provided, the first one will be used.
- The SAMPLE.PROJECT.NAME.ALL list will include the control project name.

Using HIDE to Exclude Empty Columns

You can use tthe HIDE metadata element to optionally hide a column if it contains no data. The following lines in the metadata will hide a data column when empty:

```
HIDE, ${OUTPUT.UDF.SAMPLEUDF}, IF, NODATA
```

Assuming ${OUTPUT.UDF.SAMPLEUDF} is one of the data columns specified in the template, then that column will be hidden whenever there is no data to show in the output file. If a list of fields is provided, then any empty ones will be hidden:

```
HIDE, ${OUTPUT.UDF.SAMPLEUDF},${PROCESS.TECHNICIAN}, ${PROCESS.LIMSID}, IF,
NODATA
```

You may also hide only one representation of a specific column or field:

```
HIDE, ${PROCESS.TECHNICIAN##FirstName}, IF, NODATA
```

Using HIDE to Exclude Empty HEADER rows

You can also use the HIDE metadata element with tokens in the header section. If one or more tokens are used for a header key value pair, and there are no values for any of the tokens, the entire row will be hidden.

Assuming ${OUTPUT.UDF.SAMPLEUDF} is one of the rows specified in the template header section, that header row will be hidden whenever there is no data to display in the output file.

If a list of tokens is provided for the value, the row will only be shown if one or more of the tokens resolves to a value:

```
HIDE, ${OUTPUT.UDF.SAMPLEUDF},${PROCESS.TECHNICIAN}, ${PROCESS.LIMSID}, IF,
NODATA
```

Generating Multiple Files

If you would like to generate multiple files, you can use the following GROUP.FILES.BY metadata elements:

- GROUP.FILES.BY.INPUT.CONTAINERS
- GROUP.FILES.BY.OUTPUT.CONTAINERS

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These elements allow a file to be created per instance of the specified element in the step, for example, one file per input or per output container. Step level information appears in all files, but sample information is specific to the samples in the given container.

For example, suppose that a step has two samples - each in their own container - with a template file calling for information about process UDFs and sample names. Using this metadata will produce two files, each of which will contain:

- One sample entry
- The same process UDF information

As a best practice, we recommend storing a copy of generated files in the LIMS. To do this, you must use the quickAttach script parameter. This parameter must be used with the destLIMSID parameter, which tells the Template File Generator script which file placeholder to use. (For details, see *Script Parameters* on page 2454.)

Naming The Files

When generating multiple files, the script gathers them all into one zip file so only one file placeholder is needed regardless of how many containers are in the step.

The zip file name may be provided in the metadata as follows:

```
GROUP.FILES.BY.INPUT.CONTAINERS,<zip file name>
GROUP.FILES.BY.OUTPUT.CONTAINERS,<zip file name>
```

Inside the zip file, include any paths specified for where files should be written. An example final structure inside the zip, where the subfolders are specified using the container name token, could be as follows:

```
GROUP.FILES.BY.INPUT.CONTAINERS,MyZip.zip
MyZip.zip
    ---- Container1
        ---- SampleSheet.csv
    ---- Container2
        ---- SampleSheet.csv
```

The file naming, writing, and uploading process works as follows:

- The outputPath parameter element is required for the script. You can use this parameter to specify the path to which the generated files will be written and/or the name to use for the file. Use this in the following scenarios:
  - When the target path/name is constant.-or-
  - When the target path/name includes something that can only be passed to the script via the command line - for example, if you want to include the value of a {compoundOutputFileLuidN} in the path.

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- The OUTPUT.TARGET.DIR metadata element overrides any path provided by outputPath , but does not change the file name. Use this:When the target path includes something that can only be accessed with token templates - for example, the name of the user who ran the step.
- The OUTPUT.FILE.NAME metadata element overrides any value provided by outputPath entirely. This token determines the name of the files that are produced for each container - for example, SampleSheet.csv. It may also contain tokens to access information, such as the container name, and it may also contain a path.

If you provide all three of outputPath, OUTPUT.TARGT.DIR, and OUTPUT.FILE.NAME, the result is that outputPath is ignored and the path specified by OUTPUT.TARGET.DIR is used as the parent under which OUTPUT.FILE.NAME is created, even if OUTPUT.FILE.NAME includes a path in addition to the file name.

If you wish to only attach files to placeholders in the LIMS and do not wish to also write anything to disk, then omit OUTPUT.TARGET.DIR and provide the outputPath parameter value as ".". This will cause files to only be written to the temporary directory that is cleaned up after the automation completes.

To produce the example of MyZip.zip, you could use the following:

- Script parameters:

```
-outputPath SampleSheet.csv
-q 'true'
-destLIMSID {compoundOutputFileLuid0}
```

- Template:

```
GROUP.FILES.BY.OUTPUT.CONTAINERS,MyZip.zip
OUTPUT.TARGET.DIR,${OUTPUT.CONTAINER.NAME}
```

Rules and Constraints

- You can only use one GROUP.FILES.BY metadata element in each template file.
- To attach the files in the LIMS as a zip file, you must provide the quickAttach parameter along with the destLIMSID.
- The zip file name may optionally be specified with the GROUP.FILES.BY metadata.
- If quickAttach is used and no zip name is specified in the template, the zip will be named using the destLIMSID parameter value.
- The zip file name, file paths, and file names should not contain characters that are illegal for directories and files on the target operating system. Illegal characters will be replaced with underscores.
- If a file name is not unique to the target directory, eg, if multiple SampleSheet.csv files are being written to /my/target/path, an error will be thrown and no files written.

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- When specifying the OUTPUT.TARGET.DIR metadata element, if a token is used that may resolve to multiple values for a single path (for example, using INPUT.NAME in the path when it will resolve to multiple sample names), one value will be chosen arbitrarily for the path. For example, you may end up with /Container1/Sample1/myfile.csv when there are two samples in the container.

Template File Contents

This article describes the metadata, tokens, and special characters that you can include in your custom template files for use with the Template File Generator.

Metadata

The following table lists and describes the metadata elements that you can include in your template files.

- Unless otherwise specified, metadata elements are optional. In some cases, a metadata element must be used in conjunction with another element. For example, ILLEGAL.CHARACTERS must be used with ILLEGAL.CHARACTER.REPLACEMENTS.
- Unless otherwise specified, metadata elements can appear multiple times in the template. However, if they are paired with values, only the first occurrence is used. The other lines are silently ignored.
- Unless otherwise specified, if a metadata element requires a single value, any additional values are ignored when the file is generated. For example, suppose you include the OUTPUT.TARGET.DIR <path> metadata in your template file and provide more than one value for <path>. The script will process only the first (valid) path value and will ignore all other values.
- Unless "metadata syntax must match exactly" is specified, metadata elements are detected and used even if there is text appended before or after them. For example the following expressions are equivalent:

```
LIST.SEPARATOR, COMMA
This-expression-is-equivalent-LIST.SEPARATOR-to-the-one-above, COMMA
```

For more information on metadata and how to use metadata elements in your template files, see the *Creating Template Files* article > Metadata section.

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Metadata Element	Description
CONTROL.SAMPLE.DEFAULT.PROJECT.NAME, <project name>	Defines a project name for control samples. The value specified is used to determine the SAMPLE.PROJECT.NAME and SAMPLE.PROJECT.NAME.ALL token values. • If not specified, the default project name for control samples is left empty. • If no project name follows the metadata element, the project name is left empty.
Examples: CONTROL.SAMPLE.DEFAULT.PROJECT.NAME, My Control Sample Project See also *Creating Template Files* article > Examples section > Defining a project name for control samples	
EXCLUDE.CONTROL.TYPES, <control-type name>, <control-type name>, ...	Excludes control inputs that have a control-type uri matching an entry from the exclusion list. • The metadata entry must be followed by one or more control-type name, otherwise file generation is aborted. • Each control-type name must exist in the LIMS and the metadata syntax must match exactly. If this is not the case, file generation continues, but a warning message displays and a warning is logged in the log file. • A warning is issued if the metadata element is included more than once.
Examples: EXCLUDE.CONTROL.TYPES, PhiX v3, EXCLUDE.CONTROL.TYPES, PhiX v3, Endogenous Positive Control	
EXCLUDE.CONTROL.TYPES.ALL	Excludes all control types. Takes precedence over EXCLUDE.CONTROL.TYPES • The metadata syntax must match exactly. • If this metadata element is included more than once, file generation completes, but a warning message is logged in the log file.

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Metadata Element	Description
EXCLUDE.INPUT.ANALYTES	Excludes inputs of type Analyte (derived sample) from the generated file. If used without the INCLUDE.INPUT.RESULTFILES element, the generated files will be empty. File generation finishes with a warning message and a warning is logged in the log file.
EXCLUDE.OUTPUT.ANALYTES	Excludes outputs of type Analyte (derived sample) from the generated file. The generated file(s) will be empty if: • This element is used without the INCLUDE.OUTPUT.RESULTFILES element. • There is no per input or shared result file output analyte in the step (or container if a GROUP.FILES.BY is enabled).In both scenarios, file generation finishes with a warning message and a warning is logged in the log file.
GROUP.FILES.BY.<grouping>, <zip file name> *The following groupings are supported:* • *GROUP.FILES.BY.INPUT.CONTAINERS- generates one file per input container* • *GROUP.FILES.BY.OUTPUT.CONTAINERS- generates one file per output container*	Creates a file for each instance of the specified grouping, i.e., one file per input or per output container. The script gathers all files together into one zip file so only one file placeholder is needed. The metadata may be followed by the name of the zip file that will contain the grouped files. Otherwise, the value set by the -destLIMSID script parameter is used for the file name. The following scenarios will abort file generation: • Collisions between the file names. (See OUTPUT.FILE.NAME) • Attempting to group files by both input and output container in the same template.
Examples: GROUP.FILES.BY.INPUT.CONTAINERS, MyInputContainerFile GROUP.FILES.BY.OUTPUT.CONTAINERs, MyOutputContainerFile See also *Creating Template Files* article > Examples section > Generating multiple files.	

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Metadata Element	Description
HIDE, <token>, <token>, ... IF <case> *The following case is supported:* • *NODATA: Line/Column is removed if the token has no value.*	Removes lines from the HEADER_BLOCK section and columns from the HEADER and DATA sections when a <token> matches the <case>. • All HIDE lines in the template are treated. • There can be one or more <token> on a HIDE line. • If there is no <token> between HIDE and IF, file generation is aborted. • All tokens must be part of the supported tokens list. Unsupported tokens will abort the file generation. Otherwise, file generation is aborted.
ILLEGAL.CHARACTERS, <character>, <character>, ... ILLEGAL.CHARACTER.REPLACEMENTS, <replacement>, <replacement>, ... *<character> supports Special Character Mapping*	Specifies characters that must not appear in the generated file, and replaces them. • Each <character> is replaced by the matching <replacement> element. • Refer to Special characters section below to determine if the illegal character must be specified using a keyword. • If ILLEGAL.CHARACTERS or ILLEGAL.CHARACTER.REPLACEMENTS is missing, file generation completes with a warning message and a warning is logged in the log file. No character replacement is performed. • The lists following ILLEGAL.CHARACTERS and ILLEGAL.CHARACTER.REPLACEMENTS must match 1-to-1 in order. Otherwise, file generation completes with a warning message and a warning is logged in the log file. No character replacement is performed.
Examples: ILLEGAL.CHARACTERS,PERIOD ILLEGAL.CHARACTER.REPLACEMENTS,_	ILLEGAL.CHARACTERS,TAB,PERIOD,#,<,> ILLEGAL.CHARACTER.REPLACEMENTS,_,_,_,",'

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Metadata Element	Description
INCLUDE.INPUT.RESULTFILES	Includes inputs of type ResultFile in the generated file. (By default these are excluded.) Note: • In LIMS v4.2, ResultFile inputs are only supported in the Operations Interface and API. • In LIMS v5 and later, ResultFile inputs are only supported in the API.
INCLUDE.OUTPUT.RESULTFILES	Includes outputs of type ResultFile in the generated file. (By default these are excluded.)
LIST.SEPARATOR, <separator> *<separator> supports Special Character Mapping*	Specifies character(s) used to separate elements for tokens that return lists (e.g., SAMPLE.PROJECT.NAME.ALL). • If this metadata is not specified, COMMA is used by default. • Must be followed by the separator character(s) to be used. If no separator is specified, file generation is aborted. • Refer to the Special characters section below to determine if the character must be provided using a keyword
Example: LIST.SEPARATOR,"; "	

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Metadata Element	Description
OUTPUT.FILE.NAME, <file name>	Specifies the name for the generated file(s). • The metadata syntax must match exactly. • A subset of tokens is supported in the file name. • If the metadata element is not followed by the file name, file generation is aborted. • If this metadata element is not provided or is incomplete, the value of the -o script parameter is used instead. This causes collisions when multiple files are generated (See Script parameters > Output file). In this case, file generation completes with an exception message and a warning is logged in the log file. • Using a path in the file name is deprecated, but still supported (update to OUTPUT.TARGET.DIR). File generation finishes with a warning message and a warning is logged in the log file. • Characters in the file name must be either alpha-numeric, underscores, dashes or periods.Illegal characters are replaced (see OUTPUT.FILE.NAME.ILLEGAL.CHARACTER.REPLACEMENT) – File generation finishes with a warning message and a warning is logged in the log file.
Examples: OUTPUT.FILE.NAME,NewTemplateFileName.csv You can include 'grouping' tokens in the file name, which allows you to create unique file names when generating multiple files. For details and a list of supported tokens, see *Creating Template Files* article > Examples section > Using token values in file names.	

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Metadata Element	Description
OUTPUT.FILE.NAME.ILLEGAL.CHARACTER.REPLACEMENT, <replacement>	Specifies the character(s) to use when replacing illegal characters in file names. • Legal characters are either alpha-numeric or an underscore, dash or period. • If this metadata element is not present, an underscore is used instead. (File generation finishes with a warning message and a warning is logged in the log file.) • If the replacement character is an illegal character itself, an underscore is used. • Must be followed by the character(s) to use for replacing illegal characters in file names. Otherwise, file generation is aborted.
OUTPUT.SEPARATOR, <separator> *<separator> supports Special Character Mapping*	Specifies the character(s) used to separate columns in the output. • If this metadata is not specified, COMMA is used by default. • Must be followed by the separator character(s) to be used. If no separator is specified, file generation is aborted. • Refer to the Special characters section below to determine if the separator must be provided using a keyword
Example: OUTPUT.SEPARATOR,TAB	
OUTPUT.TARGET.DIR, <path>	Specifies the name for the generated file(s). • The metadata syntax must match exactly. • A subset of tokens is supported in the file name. • If the metadata element is not followed by a path, file generation is aborted. • If this metadata element is not provided or is incomplete, the value of OUTPUT.FILE.NAME is used instead. • If OUTPUT.FILE.NAME contains a path, it is replaced by <path>.
Example: OUTPUT.TARGET.DIR,/Users/LabTech/TemplateFiles/ See also *Creating Template Files* article > Examples section > Using token values in file names.	

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Metadata Element	Description
PROCESS.POOLED.ARTIFACTS	Includes pools in the generated file as if they were regular input artifacts. • When this metadata element is present, it uses demultiplexing logic and prints one row per sample in the pool. • In the case of a submitted pool, sample names are generated following this pattern: "<pool-name>-<reagent-id>" • If an input is not pooled in this mode, the input is treated as a pool of one sample. • If the input is a pool of pools with only one sample, then it prints out as a pooled artifact instead of an input prior to the pool. • If this metadata element is not included, if the input is a pool, it is treated as if it were a single sample and only one row is output in the file.
SCRIPT.VERSION, <major>.<minor>.<patch>	Provides the version of the compatible DriverFileGenerator.jar file. • Version compatibility is only checked if this metadata is present. • <major>.<minor>.<patch> must all be present. Otherwise, file generation is aborted. • File generation is aborted if SCRIPT.VERSION <major> does not match the Template File Generator version. • The file generation continues with a warning if the SCRIPT.VERSION is later than the Template File Generator version (<minor> and <patch> only). Ensure your NGS version is up to date.
Example: SCRIPT.VERSION,1.0.2	
SORT.BY.${token}, ${token}, ...	Sorts the <DATA> rows based on the ${token} specified. • There is no reverse order. • If the SORT.BY. metadata element is not followed by ${token}, it is silently ignored.

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Metadata Element	Description
Example: SORT.BY.${INPUT.CONTAINER.ROW}${INPUT.CONTAINER.COLUMN} See C *reating Custom Template Files* article > Sorting logic section.	
SORT.VERTICAL	Sorts the <DATA> rows based on container column placement. • SORT.BY.${INPUT.CONTAINER.ROW}${INPUT.CONTAINER.COLUMN} must also be present in the template. Otherwise SORT.VERTICAL will have no effect and is silently ignored.
Example: See C *reating Custom Template Files* article > Sorting logic section.	

Tokens

A token is a placeholder variable that is replaced with unique data at run time. You can include tokens in automation command lines, in scripts, and in template files.

For example, suppose you include the INPUT.CONTAINER.NAME token in a template file generated by a step. At run time, this token is replaced with the name of the container that was input to the step.

All tokens included in a template file must appear in the following form: ${TOKEN}, for example - ${INPUT.CONTAINER.NAME}.

Input and Output Tokens

For steps with ResultFile inputs or outputs, refer to the following entries in the Metadata table:

- INCLUDE.INPUT.RESULTFILES
- INCLUDE.OUTPUT.RESULTFILES

Token	Description
INPUT.LIMSID OUTPUT.LIMSID	The LIMS ID of a given input / output
INPUT.NAME OUTPUT.NAME	The name of a given input / output
INPUT.CONTAINER.COLUMN OUTPUT.CONTAINER.COLUMN	The column part of the placement of a given input / output in its container

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Token	Description
INPUT.CONTAINER.LIMSID OUTPUT.CONTAINER.LIMSID	The LIMS ID of the container of a given input / output Also supported in: • HEADER_BLOCK • file name
INPUT.CONTAINER.NAME OUTPUT.CONTAINER.NAME	The name of the container of a given input / output Also supported in: • HEADER_BLOCK • file name
INPUT.CONTAINER.PLACEMENT OUTPUT.CONTAINER.PLACEMENT	The placement of a given input / output in its container. Format defined in the <PLACEMENT> segment
INPUT.CONTAINER.ROW OUTPUT.CONTAINER.ROW	The row part of the placement of a given input / output in its container
INPUT.CONTAINER.TYPE OUTPUT.CONTAINER.TYPE	The type of container holding a given input / output Also supported in: • HEADER_BLOCK • file name
INPUT.CONTAINER.UDF.<udf name> OUTPUT.CONTAINER.UDF.<udf name>	Get the value of a UDF on the container of a given input / output
INPUT.REAGENT.CATEGORY OUTPUT.REAGENT.CATEGORY	List of categories of reagent on a given input / output
INPUT.REAGENT.NAME OUTPUT.REAGENT.NAME	List of reagents on an input / output
INPUT.REAGENT.SEQUENCE OUTPUT.REAGENT.SEQUENCE	List the sequence of each category of reagent on a given input / output
INPUT.UDF.<udf name> OUTPUT.UDF.<udf name>	Get the value of a UDF on a given input / output
INPUT.POOL.NAME	If the current input is a pool, provides its name. Empty if the input is not a pool
INPUT.POOL.PLACEMENT	If the current input is a pool, provides its placement (not affected by the <PLACEMENT> section) Empty if the input is not a pool
INPUT.POOL.UDF.<udf name>	If the current input is a pool, provides one of its UDFs Empty if the input is not a pool.

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Process Tokens

Token	Description
PROCESS.LIMSID	The LIMS ID of the current process Also supported in: • HEADER_BLOCK • file name
PROCESS.NAME	The name of the current process
PROCESS.UDF.<udf name>	Get the value of a process UDF (on the current step) Also supported in: • HEADER_BLOCK • file name
PROCESS.TECHNICIANSee Upgrade note in *Creating Template Files* article.	The first and last name of the technician running the current process Also supported in: • HEADER_BLOCK • file name

Submitted Sample Tokens

Token	Description
SAMPLE.LIMSID	List of all submitted sample LIMS IDs of a given artifact
SAMPLE.NAME	List of all submitted sample names of a given artifact
SAMPLE.UDF.<udf name>	Get the value of a UDF for the project containing the submitted samples of a given artifact
SAMPLE.UDT.<udt name>.<udf name>	Get the value of a UDT UDF on the submitted samples of a given artifact
SAMPLE.PROJECT.CONTACT	List of the project contacts for all submitted samples of a given artifact
SAMPLE.PROJECT.CONTACT.ALL	List of the project contacts for the submitted samples of all artifacts Prints all unique project contact names in a line (first name followed by last name) separated by LIST.SEPARATOR Also supported in: • HEADER_BLOCK • file name

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Token	Description
SAMPLE.PROJECT.LIMSID	List of the project LIMS IDs for all submitted samples of a given artifact
SAMPLE.PROJECT.NAME .	List of projects for all submitted samples of a given artifact (uses CONTROL.SAMPLE.DEFAULT.PROJECT.NAME)
SAMPLE.PROJECT.NAME.ALL	List of projects for the submitted samples of all artifacts (uses CONTROL.SAMPLE.DEFAULT.PROJECT.NAME) Prints all unique project names in a line, separated by LIST.SEPARATOR Also supported in: • HEADER_BLOCK • file name
SAMPLE.PROJECT.UDF.<udf name>	Get the value of a UDF for the project containing the submitted samples of a given artifact. Example: ${SAMPLE.PROJECT.UDF.My Project Level Field}

Other Tokens

Token	Description
DATE	Current date (i.e., when the script is run). The default format uses the host's locale setting.
INDEX	Row number of the data row (in <DATA> segment), starting from 1

Special Characters

CSV and template file generation special characters have substitution symbols within templates.

Substitution Symbol	Character Represented
ASTERISK	*
BACKSLASH	\
CARET	^
CLOSING_BRACE	}
CLOSING_BRACKET	]
CLOSING_PARENTHESIS	)

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Substitution Symbol	Character Represented
COMMA	,
DOLLAR_SIGN	$
DOUBLE_QUOTE	"
OPENING_BRACE	{
OPENING_BRACKET	[
OPENING_PARENTHESIS	(
PERIOD	.
PIPE	|
PLUS_SIGN	+
QUESTION_MARK	?
SINGLE_QUOTE	'
TAB	tab

Troubleshooting Template File Generator

When the Template File Generator generates the file, it creates a log file and attaches it to the step in the LIMS.

If the file generation process encounters errors, these error conditions appear in the log file.

If the file generation process completes successfully, the log file contents resemble the following example:

The external script driver_file_generator has completed successfully:

```
Template simpleTemplate.csv has been found
Attempting to load input artifacts..........Success
Attempting to load output artifacts.........Success
Attempting to load output containers........Success
WARNING: </FOOTER> segment was not found. The <FOOTER> will be skipped
No illegal character specifications have been found in the template
```

When troubleshooting Template File Generator issues, you will find detailed information in the Automation Worker log files.

Getting Started - Sample Input Sheet Generator

Compatibility: NGS v5.16 and earlier

The `driver_file_generator.groovy` script uses a supplied template file to produce a corresponding sample input sheet file. This enables the user to produce custom sample input sheet files through the creation of an appropriate template file, without requiring scripting or development resources.

Note that while the output format of the template file is not limited to CSV, this is the most common sample sheet file format encountered.

Most CSV-based sample input sheet files follow the same basic structure: metadata, header block, header, data, footer. (For details, see *Sample Input Sheet Template Sections* on page 2486.)

How it Works

1. The script is initiated via EPP/automation.
2. The script uses a CSV template file to create a sample input sheet file, based on the specifications within the template.
3. The script then extracts data from the LIMS via the API based on tokens defined as ${TOKENTEXT} within the template. (The available tokens are described in *Creating Sample Input Sheet Templates* on page 2490.)

   A few special options are available for inclusion in the template. These do not directly pull data from the API. Instead, they modify what has been gathered in the sample sheet file. These options are outlined under the Metadata section in *Sample Input Sheet Template Sections* on page 2486.

4. The script parses the comma-delimited template file and processes the five sections this file may contain: metadata, header block, header, data, and footer, each of which is enclosed inside tags.

For example:

```
<HEADER_BLOCK>Header block entry one, two, three More information </HEADER_
BLOCK>
```

Rules and Constraints

If any of the following conditions is not met - the tag, and everything inside of it, will be ignored by the script and a warning will display in the log file:

- With the exception of the metadata, all template sections must be enclosed inside tags.
- Each tag must have its own line, and must be the only tag present on that line.
- No other entries, even empty ones, are allowed.
- All opened tags must be closed.

Sample Input Sheet Template Sections

Compatibility—NGS v5.16 and earlier

A generic sample input sheet file template may consist of up to seven different sections. These sections are described below.

1) Metadata

The metadata describes information about the file that is not retrieved from the API, such as the file output directory to use and how the data contents should be sorted.

- Metadata is not strictly confined to a section, and is not designated by surrounding tags.
- Each piece of metadata information must appear on a separate line from the rest of the text in the template file.
- Metadata entries can be anywhere in the template. However, it is the recommended best practice to group them either at the top or the bottom of the file.

2) Header Block

The header block consists of information, which may include plain text and data from the LIMS, that does not appear multiple times in the file (e.g. it is not part of the data rows).

- To include a header block in the template, enclose it within the `<HEADER_BLOCK>` and `</HEADER_BLOCK>` tags.
- Tokens that may have more than one value are not fully supported in the header block. If one of these is encountered, such as `SAMPLE.NAME`, a warning will be logged and the first value retrieved from the API will be used.

For example, using `INPUT.CONTAINER.TYPE` in the header block when there is only one type of input container would simply result in that type being present in the output sample sheet file. However, if there were more than one type of input container, only the first one encountered while processing the data would appear.

Fully-supported tokens:

- PROCESS.TECHNICIAN
- PROCESS.LIMSID
- PROCESS.UDF

UDF token usage:

To include a UDF token in a template, specify the token as `${PROCESS.UDF.process udf name}`, where 'process udf name' is the exact name of the UDF within the LIMS. UDF names with spaces are supported.

3) Header

The header section describes the header line of the sample sheet file's data section. A simple example may be "Sample ID,Placement".

- This section of the template starts with `<HEADER>` and ends with `</HEADER>`.
- The header can only include plain text entries; no tokens are allowed.

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4) Data

The data section of the template describes the contents of the rows that will contain unique values pulled from each input/output to the process.

- This section of the template is enclosed in `<DATA>` and `</DATA>` tags.
- The data section allows both tokens and text entries, and any tokens are allowed.
- If there is at least one token relevant to the process inputs or outputs, this section will produce a row for each `PerInput`entry in the process input-output-map. If no `PerInput` entries are present in the process input-output-map, the script will attempt to add data rows for `PerAllInputs` entries.
- Only unique data rows are written to the sample sheet file. As such, the tokens must provide distinctive enough data (i.e. something more than just CONTAINER.NAME) if all of the input-output entry pairs are desired in the generated driver file.
- By default, the script processes only sample entries; however, there are metadata options that allow inclusion of result files and exclusion of samples.
- Metadata sorting options are applied to this section of the sample sheet file only. Artifacts are loaded dynamically and only once, if the tokens that need them have been encountered in the template file.
- Input and Output artifacts are always loaded if `<DATA>` section is present in the template, due to the need to determine what type of artifacts the script is dealing with.
- Pooled artifacts are treated as a single input artifact. (It is possible to expand the script to process each pooled artifact separately should such a need arise.)

5) Footer

The footer section is very similar to the HEADER section.

- This section is enclosed in `<FOOTER>` and `</FOOTER>` tags.
- No tokens are allowed.

6) Placement definition

- This section is enclosed in `<PLACEMENT>` and `<PLACEMENT>` tags.
- The section specifies the way `INPUT.CONTAINER.PLACEMENT` and `OUTPUT.CONTAINER.PLACEMENT` tokens are formatted.
- Within the segment valid groovy code must be present (as if it was a separate script).

The inputs to the script are:

- String row
- String column
- Node containerTypeNode

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The script must return a string, which replaces the corresponding <PLACEMENT> tag in the template.

Logic within the placement tags can be as complex as needed, as long as it can be compiled by a groovy compiler.

Example

In the following example:

- If the container type is a "96 well plate," sample placement A1 will return as " A_1 "
- If the container type is not a "96 well plate," sample placement A1 will return as " A:1 "

```
<PLACEMENT>
   // The inputs to this segment are: String row, String column, Node
   containerTypeNode
   if (containerTypeNode.@name == "96 well plate")
      return row + "_" + column
   else
      return row + ":" + column
</PLACEMENT>
```

Zero Padding Example

```
<PLACEMENT>
   // The inputs to this segment are: String row, String column, Node
   containerTypeNode
   String zeroPad (String entry) {
      if (entry.isNumber() && entry.size() == 1) return "0" + entry
      return entry
   }
   return zeroPad(row) + ":" + zeroPad(column)
</PLACEMENT>
```

7) Custom Token Format Definition

This section defines logic that should be applied to specific tokens in order to change the format in which they appear in the final document produced.

It is enclosed in <TOKEN_FORMAT></TOKEN_FORMAT> tags and supports groovy syntax. The code written is applied to its corresponding token, and then replaces that token's value with the result once the code is evaluated.

The inputs to the script are:

- String token

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A variable "token" will be available and set to the current value retrieved from the LIMS. The code is applied to each instance of the token.

Token Format Example: Technician Name

In the example below, a custom format has been defined for displaying the name of the technician who ran the process.

The name of the token appears at the beginning of the Groovy code that will then be applied. In this code, the variable "token" refers to the token being affected. The return value is what will replace all instances of this token in the file.

```
<TOKEN_FORMAT>${PROCESS.TECHNICIAN}
    def name = token.split(" ")
    return "First name: " + name[0] + ", Last name: " + name[1]
</TOKEN_FORMAT>
```

Token Format Example: Container Names

In this second example, special formatting is required for two tokens. When applying logic to more than one token, they all appear inside the same set of tags.

This example appends a string to the end of the input and output container names.

```
<TOKEN_FORMAT>
    ${INPUT.CONTAINER.NAME}
    return token + "-SourcePlateName"

    ${OUTPUT.CONTAINER.NAME}
    return token + "-DestinationPlateName"
</TOKEN_FORMAT>
```

Token Format Example: Static Text Entry on Each Row

In this example, instead of making a Token, text can be entered into the content of the row. Using this example: "${OUTPUT.LIMSID}, 1" in the <DATA> section, results in each row have the LIMSID in the first column, and a 1 in the second column.

Creating Sample Input Sheet Templates

Compatibility—NGS v5.16 and earlier

New in NGS 5.4—Input and output container UDF tokens



Before creating a sample input sheet template, ensure that you understand the sections of the template - as described in *Sample Input Sheet Template Sections* on page 2486

The sample input sheet template is a comma-delimited CSV file. Its format must follow a few basic rules:

- Metadata entries must each appear on a new line and be the only entry on that line. Metadata entries must not appear inside tags.

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- Opening and closing tags must appear exactly as they are outlined below, on a new line and as the only entry on that line.
- Each opened tag must be closed, otherwise it is skipped by the script.
- The order of tags must be preserved: <HEADER_BLOCK>, <HEADER>, <DATA>, <FOOTER>
- Any sections (opening tag + closing tag combination) can be omitted from the template file.
- Entries that are separated by commas in the template will be delimited by the metadata-specified separator (default: COMMA) in the sample input sheet.
- White space is allowed in the template. However, if there is a blank line inside a tag, it will also be present in the sample input sheet produced.
- If an entry in the template is enclosed in double quotes it will be imported as a single entry and written to the sample input sheet as such, even if it has commas inside.
- To include double-quotes (or single-quotes) in the sample input sheet, use theescape character: Example: \" or \'
- To include an escape character in the sample input sheet, use two escape characters inside double-quotes. For example if you want to see \\Share\Folder\Filename.txt use "\\\\Share\\Folder\\Filename.txt" as the token.

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Template File Examples

Figure 65 Example 1: Metadata, Sample In/Sample Out Process Type

```
ILLEGAL.CHARACTERS,a,b,TAB
ILLEGAL.CHARACTER.REPLACEMENTS,A,B," "
SORT.BY.${INPUT.CONTAINER.ROW}${INPUT.CONTAINER.COLUMN}
SORT.VERTICAL
<HEADER_BLOCK>
First line of text
Project names involved: ${SAMPLE.PROJECT.NAME.ALL}
Process limsid = ${PROCESS.LIMSID}
Technician is ${PROCESS.TECHNICIAN}
</HEADER_BLOCK>
<HEADER>
[Sample Setup]
Input Container Placement, Sample Name, Output Container Placement, Project Name of the Sample, Index
</HEADER>
<DATA>
${INPUT.CONTAINER.PLACEMENT},${INPUT.LIMSID},${OUTPUT.CONTAINER.PLACEMENT},${OUTPUT.LIMSID},${INDEX}
</DATA>
<PLACEMENT>
if (containerTypeNode.@name == "96 well plate") return row + "_" + column
else return row + ":" + column
</PLACEMENT>
<TOKEN_FORMAT>
${PROCESS.TECHNICIAN}
def name = token.split(" ")
return "First name: " + name[0] + "Last name: " + name[1]

${INDEX}
return "<" + token + ">\""
</TOKEN_FORMAT>

SCRIPT.VERSION,1.0.2
```

Figure 66 Example 2: Sample In/ResultFile Out Process Type

```
INCLUDE.OUTPUT.RESULTFILES
SORT.BY.${OUTPUT.LIMSID}
<HEADER_BLOCK>
Simple Header Block
</HEADER_BLOCK>
<HEADER>
Output LimsID, Position, Input Name, Input LimsID
</HEADER>
<DATA>
${OUTPUT.LIMSID},${OUTPUT.CONTAINER.ROW}:${OUTPUT.CONTAINER.COLUMN},${INPUT.NAME},${INPUT.LIMSID}
</DATA>
```


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Figure 67 Example 3: Sample In/Shared ResultFile Out Process Type

```
INCLUDE.OUTPUT.RESULTFILES
<HEADER>
Artifact Name, Artifact LimsID, Position
</HEADER>
<DATA>
${INPUT.NAME},${INPUT.LIMSID},${INPUT.CONTAINER.LIMSID},${INPUT.CONTAINER.ROW}:${INPUT.CONTAINER.COLUMN}
</DATA>
```

Tokens

All of the tokens below must appear in the template in the following form: ${TOKEN}.

Example: ${PROCESS.LIMSID}

If your process contains ResultFile inputs or outputs, please refer to the Metadata section, specifically INCLUDE.INPUT.RESULTFILES and INCLUDE.OUTPUT.RESULTFILES.

Input and OutputTokens

Input Tokens	Output Tokens
INPUT.LIMSID	OUTPUT.LIMSID
INPUT.NAME	OUTPUT.NAME
INPUT.CONTAINER.NAME	OUTPUT.CONTAINER.NAME
INPUT.CONTAINER.LIMSID	OUTPUT.CONTAINER.LIMSID
INPUT.CONTAINER.TYPE	OUTPUT.CONTAINER.TYPE
INPUT.CONTAINER.ROW	OUTPUT.CONTAINER.ROW
INPUT.CONTAINER.COLUMN	OUTPUT.CONTAINER.COLUMN
INPUT.CONTAINER.PLACEMENT	OUTPUT.CONTAINER.PLACEMENT
INPUT.CONTAINER.UDF.name	OUTPUT.CONTAINER.UDF.name
INPUT.UDF.<UDF NAME>	OUTPUT.UDF. <UDF NAME>
INPUT.REAGENT.NAME	OUTPUT.REAGENT.NAME
INPUT.REAGENT.CATEGORY	OUTPUT.REAGENT.CATEGORY
INPUT.REAGENT.SEQUENCE	OUTPUT.REAGENT.SEQUENCE
INPUT.POOL.NAME	
INPUT.POOL.PLACEMENT	
INPUT.POOL.UDF. <UDF NAME>	

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Process Tokens

Process Tokens	Submitted Sample Tokens
PROCESS.LIMSID	SAMPLE.LIMSID
PROCESS.NAME	SAMPLE.NAME
PROCESS.TECHNICIAN	SAMPLE.PROJECT.CONTACT
PROCESS.UDF.<UDF NAME>	SAMPLE.UDF. <UDF NAME>
	SAMPLE.PROJECT.NAME
	SAMPLE.PROJECT.LIMSID
	SAMPLE.UDT. <UDT NAME> . <UDF NAME>

Misc Tokens

Misc Tokens	Description
INDEX	Row number of the data row, index starting from 1
DATE	Current date (i.e., when the script is being run)
SAMPLE.PROJECT.NAME.ALL	Prints all unique project names in a line, separated by LIST.SEPARATOR
SAMPLE.PROJECT.CONTACT.ALL	Prints all unique project contact names in a line (first name followed by last name) separated by LIST.SEPARATOR

Metadata

Each metadata entry must be on a separate line. These are not data tokens, so they do not need to be enclosed in tags.

When using OUTPUT.FILE.NAME, you may wish to give the file a name that does not begin with the LIMSID of the placeholder it will be attached to in the LIMS. To still attach this file, the quickAttach and destLIMSID parameters may be used in the EPP configuration for the sample input sheet file generation.

Name of Metadata Entry	Description
SCRIPT.VERSION	Version of the compatible DriverFileGenerator.jar file, please include this entry into your templates. Example: SCRIPT.VERSION,1.0.2

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Name of Metadata Entry	Description
OUTPUT.TARGET.DIR	Provide this token to specify the directory in which to write the sample input sheet file . If the directory or its parent directories do not exist, they will be created. Example: OUTPUT.TARGET.DIR,/Users/LabTech/DriverFiles/
OUTPUT.FILE.NAME	Provide this token to rename the sample input sheet with the specified name. Example: OUTPUT.FILE.NAME,NewDriverFileName.csv
OUTPUT.SEPARATOR	The sample input sheet will be delimited with the specified separator. The default sample input sheet delimiter used is COMMA. Refer to the *Special Characters* on page 2496 section of this page to determine if the separator must be provided using a keyword. Example: OUTPUT.SEPARATOR,TAB
LIST.SEPARATOR	Tokens that return lists (e.g., SAMPLE.PROJECT.NAME.ALL) will be separated by the specified String. Example: LIST.SEPARATOR,"; "
ILLEGAL.CHARACTERS	Specify characters that must not appear in the file produced. Each illegal character will be replaced with its corresponding replacement specified by the replacements token. Refer to the *Special Characters* on page 2496 section of this page to determine if the illegal character must be specified using a keyword. Example: ILLEGAL.CHARACTERS,TAB,PERIOD,#,<,>,etc
ILLEGAL.CHARACTER.REPLACEMENTS	Provides the replacement policy for each illegal character. The number of replacements provided must match the number of specified illegal characters (If only one replacement character is specified, it will be used to replace all illegal characters); replacement will be performed in corresponding order. Example: ILLEGAL.CHARACTER.REPLACEMENTS, _,",",_,_,_,_ ILLEGAL.CHARACTER.REPLACEMENTS,_

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Name of Metadata Entry	Description
SORT.BY.	Specify tokens by which the data will be sorted alphanumerically within the sample input sheet file. More than one token can be provided e.g., to sort by container placement by row follow the usage example to the right, to sort by placement with multiple containers present provide the container name token as a sort option as well. For more information, see *Sorting Logic* on page 2497. Example: SORT.BY.${INPUT.CONTAINER.ROW}${INPUT.CONTAINER.COLUMN}
SORT.VERTICAL	Sort data by container placement by column. SORT.BY.${INPUT.CONTAINER.ROW}${INPUT.CONTAINER.COLUMN} must be present in the template as well, otherwise SORT.VERTICAL will have no effect. For more information, see *Sorting Logic* on page 2497. Example: SORT.VERTICAL
PROCESS.POOLED.ARTIFACTS	Include artifacts stored in pools into the sample input sheet generation as if they were regular input artifacts. Example: PROCESS.POOLED.ARTIFACTS
INCLUDE.INPUT.RESULTFILES	Includes input ResultFiles in the sample input sheet file. (By default these are excluded.) Example: INCLUDE.INPUT.RESULTFILES
INCLUDE.OUTPUT.RESULTFILES	Includes output ResultFiles in the sample input sheet file. (By default these are excluded.) Example: INCLUDE.OUTPUT.RESULTFILES
EXCLUDE.INPUT.ANALYTES	Excludes analyte (sample) inputs from the sample input sheet file. (By default these are included.) Example: EXCLUDE.INPUT.ANALYTES
EXCLUDE.OUTPUT.ANALYTES	Excludes analyte (sample) outputs from the sample input sheet file. (By default these are included.) Example: EXCLUDE.OUTPUT.ANALYTE

Special Characters

Keyword	Character Represented
COMMA	,

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Keyword	Character Represented
TAB	tab
PERIOD	.
BACKSLASH	\
OPENING_BRACE	{
CLOSING_BRACE	}
DOLLAR_SIGN	$
SINGLE_QUOTE	'
DOUBLE_QUOTE	"
PIPE	|
OPENING_PARENTHESIS	(
CLOSING_PARENTHESIS	)
OPENING_BRACKET	[
CLOSING_BRACKET	]
QUESTION_MARK	?
ASTERISK	*
PLUS_SIGN	+
CARET	^

Sorting Logic

Sorting in the file is done either alphanumerically or by vertical placement information. To sort vertically, include the token SORT.VERTICAL in the metadata section of the template file.

The following sorting metadata must also be included in the file:

```
SORT.BY.${OUTPUT.CONTAINER.ROW}${OUTPUT.CONTAINER.COLUMN}
```

At this point any SORT.BY tokens will be sorted using the vertical sorter instead of the alphanumeric sort.

An important thing to note about sorting is that it must be done using a combination of sort keys (provided to SORT.BY) that always produce a unique value in the file.

For example, sorting by just OUTPUT.CONTAINER.NAME would not work for samples in 96 well plates, but would work for samples placed in tubes.

To apply sorting to samples in 96 well plates you could narrow the sort key to a unique combination such as:

```
SORT.BY.${OUTPUT.CONTAINER.NAME}${OUTPUT.CONTAINER.ROW}${OUTPUT.CONTAINER.COLUMN}
```

Sorting behavior on non-unique combinations is not guaranteed to be predictable.

Sample Input Sheet Generator EPP

Compatibility—NGS v5.16 and earlier

New in NGS 5.4—quickAttach and destLIMSID parameters

Once you have defined a sample input sheet template, you must link it to a generic sample input sheet file generation EPP call. The EPP string can then be attached to your desired process.

Parameters

The following table defines the parameters used by the `driver_file_generator` script:

-i {processURI}	LIMS process URI (Required)
-u {username}	LIMS username (Required)
-p {password}	LIMS password (Required)
-t {templatePath}	Template file path (Required)
-o {outputPath}	Output sample input sheet file path (Required)
-l {logFileName}	Log file name (Required)
-q {quickAttach}	Boolean value. Provide as 'true' to attach the file on script completion. To attach otherwise, name the file starting with the placeholder LIMSID. Default is false. (Optional) *(NGS 5.4)*
-destLIMSID {destLIMSID}	LIMSID of the output to attach the sample input sheet file to - use with quickAttach (Optional) *(NGS 5.4)*

When using quickAttach, the destination LIMSID parameter must also be provided. The script will log an error and stop execution otherwise. If the destination LIMSID is provided without using quickAttach, it will be ignored. The filename for this Output file can be defined in the Sample Input Sheet Generator.

Usage

```
groovy driver_file_generator.groovy -i {processURI} -u {username} -p {password} -t {templatePath} -o {outputPath} -l {logFileName}
```

```
java -jar DriverFileGenerator-1.0.3-jar-with-dependencies.jar driver_file_generator -i {processURI} -u {username} -p {password} -t {templatePath} -o {outputPath} -l {logFileName}
```

EPP String Example

```
java -cp $ABSOLUTE_PATH/DriverFileGenerator.jar driver_file_generator -i
{processURI} -u {username} -p {password} -t {template file path} -o
{compoundOutputFile0}.csv -l {compoundOutputFile1}.html
```

LIMS v3.5 or earlier:

```
bash -c "/usr/bin/java -cp /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar driver_file_generator -i
{processURI:v2:http} -u {username} -p {password} -t
/opt/gls/clarity/customextensions/Robot.csv -o {compoundOutputFileLuid0}.csv -l
{compoundOutputFileLuid2}"
```

LIMS v4.0 or later:

```
bash -c "/opt/gls/clarity/bin/java -cp /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar driver_file_generator -i
{processURI:v2:http} -u {username} -p {password} -t
/opt/gls/clarity/customextensions/Robot.csv -o {compoundOutputFileLuid0}.csv -l
{compoundOutputFileLuid2}"
```

Example Sample Input Sheet

The following sample input sheet is created by the template example provided above.

```
* First line of text
* Process type = GEN-FWX-120404-24-101193:FrAnk Worsley
* Process UDF = Process UDF VAlue
* Second Process UDF = Second Process UDF VAlue
HeAder1,HeAder2,HeAder3
01:01,AutomAtedTestSAmple-1970041840,A:01
01:01,AutomAtedTestSAmple1163460860,B:01
01:01,AutomAtedTestSAmple-848968223,C:01
01:01,AutomAtedTestSAmple1298043294,D:01
01:01,AutomAtedTestSAmple1050639691,A:02
01:01,AutomAtedTestSAmple-126471685,B:02
01:01,AutomAtedTestSAmple2020755473,C:02
01:01,AutomAtedTestSAmple1235057387,A:03
01:01,AutomAtedTestSAmple413478423,B:03
01:01,AutomAtedTestSAmple-928783487,C:03
```

UDT and UDF names must not contain periods.

Example with Quick Attachment

```
bash -c "/opt/gls/clarity/bin/java -cp /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar driver_file_generator -i
{processURI:v2:http} -u {username} -p {password} -t
/opt/gls/clarity/customextensions/Robot.csv -q true -o {compoundOutputFileLuid0}
```

```
-l {compoundOutputFileLuid2}"
```

This attaches the file created by the generator to the placeholder for {compoundOutputFileLuid0}, with a name defined by the setting "OUTPUT.FILE.NAME" in the template file.

Sample Input Sheet Generator Error Logging

Compatibility—NGS v5.16 and earlier

Once the Sample Input Sheet Generator EPP has completed, an error log is created. This log will detail the success or failure of the process.

Below is a sample success log.

The external script driver_file_generator has completed successfully:

```
Template simpleTemplate.csv has been found
Attempting to load input artifacts..........Success
Attempting to load output artifacts.........Success
Attempting to load output containers........Success
WARNING: </FOOTER> segment was not found. The <FOOTER> will be skipped
No illegal character specifications have been found in the template
```

The following error conditions are caught by the script and appear in the log file attached in the LIMS.

Condition	Message	Severity
Provided -q without -destLIMSID.	The quick attach option has been provided without a corresponding destination LIMSID. Please provide -destLIMSID for use with -quickAttach (-q).	Error
Provided -destLIMSID without -q.	A destination LIMSID, '$destLIMSID', has been provided without the quickAttach (-q) parameter. This LIMSID will be ignored when producing the output file.	Warn
Opening tag of a segment was not found in the template	INFO: $segName segment was not found. The name must match one of the allowed tags exactly and no commas are allowed on this line	Info
Closing tag of a segment was not found in the template	INFO: $segCloseTag segment was not found. The $segName will be skipped	Info

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Condition	Message	Severity
An sample or result file artifact is being skipped by the script	INFO: limsid/limsid io-map entry with types: $inputType/$outputType is skipped. If this is a mistake, please add INCLUDE.INPUT.RESULTFILES or INCLUDE.OUTPUT.RESULTFILES as needed	Info
Token is not supported for the current segment (not in the list of the allowed tokens)	WARNING: $token is not supported for <HEADER_BLOCK>. Only the first entry will be used	Warn
The code within <PLACEMENT> tags could not be compiled	WARNING: The <PLACEMENT> code could not be compiled. Please review: $exceptionMessage	Warn
The code within <PLACEMENT> tags threw an exception	WARNING: A $e.className() exception has occurred while applying <PLACEMENT> format	Warn
The code within <TOKEN_FORMAT> tags could not be compiled	WARNING: The <TOKEN_FORMAT> code could not be compiled. Message: "message"	Warn
The code within <TOKEN_FORMAT> tags threw an exception	WARNING: A "className" exception has occurred while trying to apply <TOKEN_FORMAT> format: "exception message"	Warn
Token is not supported for the ResultFile type of input artifact (CONTAINER tokens)	WARNING: $token is not supported for ResultFile input type, limsid: limsid. Replacing with an empty String	Warn
Token is not supported for the ResultFile type of output artifact (CONTAINER tokens)	WARNING: $token is not supported for ResultFile output type, limsid: limsid. Replacing with an empty String	Warn
Token does not exist	WARNING: $token is not a supported token - it will be written to the driver file as text	Warn

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Condition	Message	Severity
Token value is null	WARNING: Token $upperCaseToken returned NULL for io-map entry with input URI: $inputURI and output URI: $outputURI. This may be a result of the <TOKEN_FORMAT> section or the artifact not having the required information$LINE_TERMINATOR"	Warn
Token extraction from the LIMS threw any Exception for any reason	WARNING: Cannot retrieve $token for input-output-map PerInput entry number $index	Warn
UDF with the specified name does not exist for the current node	WARNING: $udfName does not exist for artifact node with uri: $uri	Warn
UDT with the specified name does not exist for the current node	WARNING: $udfName does not exist for artifact node with uri: $uri	Warn
Container and placement information could not be retrieved for the given input/output artifact	WARNING: Could not retrieve container information	Warn
Specified directory does not exist when attempting to write sample input sheet to the location	WARNING: DriverFile parent directory does not exist, attempting to create required directories.....Success	Warn
ILLEGAL.CHARACTER.REPLACEMENTS metadata entry was not found after ILLEGAL.CHARACTERS entry was	WARNING: No ILLEGAL.CHARACTER.REPLACEMENTS entry have been found to match ILLEGAL.CHARACTERS. Please specify a replacement for each illegal character in the template	Warn
Number of ILLEGAL.CHARACTER.REPLACEMENTS does not match the number of ILLEGAL.CHARACTERS	WARNING: The number of illegal characters: X does not match the number of illegal character replacements: Y in the template file (And the number of illegal char replacements does not equal to 1)	Warn

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Condition	Message	Severity
LIST.SEPARATOR is one of the illegal characters	WARNING: $LIST_SEPARATOR will be replaced because it appears in the ILLEGAL.CHARACTERS entry, please choose a different LIST.SEPARATOR value if you do not want it to get replaced	Warn
Minor or point script version number is outdated	WARNING: The template expects DriverFileGenerator version $scriptVersionReq, but current version is: $scriptVersion. Please check the compatibility and update accordingly$LINE_TERMINATOR"	Warn
Script version is incompatible	ERROR: The template expects DriverFileGenerator version $scriptVersionReq, but current version is: $scriptVersion. Please obtain a compatible version of the DriverFileGenerator.jar from the BaseSpace Clarity LIMS Support team.	Error
Writing the sample input sheet failed	ERROR: Failed to write driver file to $driverFilePath because $ExceptionMessage	Error

Add Blank Lines

Compatibility: BaseSpace Clarity LIMS v3.0.0 or later; NGS Extensions Package v5.2.0 or later

Some instruments, such as Bioanalyzer and the ABI 7900HT, require input files that include a line for every well in a container.

Currently, files created with the file generator can only contain data lines for each well that is in use and cannot add lines for empty wells. This extension script allows you to process a file in a certain format to add customizable lines for the empty container wells.

The Script

The addBlankLines script allows for the creation of files that require a line entry for every well in a container, including those wells that are empty.

To accomplish this, the script takes in an existing file, created with file generator, and processes it to add the new lines.

1. The script reads the file and separates it into header, data, and footer sections.

2. Using the API, the script obtains the container size, its unavailable wells, and the placement of each sample.
3. Using this information, the script runs through each possible placement and builds a full set of data. This includes a line for each empty well, in addition to all original lines of data. If desired, a line for each unavailable well may also be included using script parameters described.
4. The file is then rewritten with the header, full data, and footer.

> The addBlankLines script overwrites the input file provided, replacing it with the new file that includes the additional container line entries.

Placement information is written using a numeric index that is converted from the LIMS well placement. The line information that is inserted for an empty well is provided to the script. This is controlled by the blankLineString parameter, described.

The logic that determines whether the script uses input samples and containers or output samples and containers is as follows:

- By default the script will use outputs.
- If there are no outputs or if the optional parameter -forceInputs true is configured, the script will use inputs.

Script Parameters and Usage

The following table defines the parameters used by the addBlankLines script.

-i {URI}	LIMS step URI (Required)
-u {username}	The LIMS username (Required)
-p {password}	The LIMS password (Required)
-f {inputFileName}	The input file name (Required)
-l {logFileName}	The log file name (Required)
-h {headerRows}	The number of lines in the header (Required)
-sep {separator}	The separator to use for the file - COMMA and TAB supported (Required)
-b {blankLineString}	The string to be used for blank lines (Required)
-c {columnType}	The data type specified in the first column of the data - LIMSID and PLACEMENT supported (Required)
-pre {indexPrefix}	The prefix to be included before the index of each well in the file output (Optional)

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-forceInputs {forceInputs}	Provide as "true" to force the script to use the input samples and container when processing, even if output samples and containers exist. (Optional)
-countUnavailable	Provide as "true" to include unavailable wells in the placement index count (Optional)
-addUnavailable	Provide as "true" to include unavailable wells as entries in the updated file (Optional)

columnType

The script uses this parameter to match each row to a sample in the LIMS. The parameter tells the script what data type to expect in the first column of any data row. Supported data types are PLACEMENT and LIMSID. For example:

```
-c LIMSID
```

- LIMSID - The script reads the LIMS ID in the first column of data, matches it directly to the sample, and uses this to get the placement of that sample through the API. It does not replace this entry in the data, however.
- PLACEMENT - The script reads the sample placement in the first column of data, then finds the sample in the container at that location. The script then replaces this placement entry with an entry of the form "{index-prefix}{index}". Supported placement formats are alphanumeric (eg A1), orcolon-separated (eg 1:1). Placements that are numeric only (eg 11) are not supported.

forceInputs

This parameter lets you force the script to use input samples and input container, even if output samples and output container are found for the step. If this parameter is not provided, the script uses output samples and output container if available; input samples and container if they are not.

indexPrefix

This parameter provides a way to place a prefix directly before the index on blank lines. If the column type is set to PLACEMENT, the indexPrefix will also be printed before the index when replacing the well placement in the data.

blankLineString

This parameter defines what is placed after the index in a blank line for an empty well. A separator character is automatically placed between the index and the blankLineString.

Any ‘ \t ’ found in the blankLineString is replaced by a tab space in the output. Otherwise, the blankLineString is left unchanged.

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Separator

The script supports tab and comma separators in the output file. These can be provided with this parameter in the form “TAB” and “COMMA”. The same separator is used to interpret the original file and produce the updated file.

countUnavailable

If provided, a container is unavailable wells will be included when determining the placement index of a well.

For example, imagine a 2x2 container with wells A:1, A:2, B:1, and B:2, where wells B:1 and B:2 are marked as unavailable. By default, the script will calculate well A:2 as well index 2, excluding the unavailable wells from the index. When the countUnavailable parameter is provided, A:2 is instead calculated as well index 3.

addUnavailable

If provided, blank lines will be added to the file for unavailable wells in addition to blank lines for unoccupied wells.

Index

The index is the well number when the wells are counted from left to right, from top to bottom. By default, unavailable wells are not counted in this index. However, if the script is configured with the countUnavailable parameter, they will be included in the index count.

Blank Line Format

The format of any blank line printed for an empty well is as follows:

```
{indexPrefix}{index}{separator}{blankLineString}
```

Rules and Constraints

The script operates with the following constraints:

- The input file and log file to update must exist locally.
- Only supports TAB and COMMA as separators in the file.
- Only supports processing one container at a time.
- Can only use the first column of the data and requires either well placement or LIMS ID as values in this column.
- Indexing always is done from left to right, top to bottom.
- The well placement format in the original file must be alphanumeric (eg A1), or separated by a colon (eg 1:1).

- As designed, the script supports processing one container only. As such, any step on which it is run must use either a single input container or a single output container, depending on which type of container you expect the script to use (see forceInputs).

Configuration

- The script should be configured to run after the creation of a file occurs - either within the same automation call or as a separate automation triggered after the call that generated the file.
- It can also be configured standalone for files that are locally accessible.

Example Automation Strings

Example 1

This example uses the addBlankLines script within the same automation call as the file generation. It also includes the optional parameter '- pre ', adding the prefix 'Sample' before the index of each well in the file output.

- The first half of the call (the section preceding the 'and and' characters) uses the file generator to create the file.
- The second half of the call (the section following the 'and and' characters) takes the generated file and processes it to add blank lines.

LIMS v3.5 or earlier:

```
bash -c "/usr/bin/java -cp /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar driver_file_generator \
   -i {processURI:v2:http} \
   -u {username} \
   -p {password} \
   -t /opt/gls/clarity/extensions/ngs-common/v5/EPP/conf/readonly/bioA_driver_
   file_template.csv \
   -o {compoundOutputFileLuid0}.csv \
   -l {compoundOutputFile1} \
&& /usr/bin/java -cp /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-
extensions.jar addBlankLines \
   -i {stepURI:v2:http} \
   -u {username} \
   -p {password} \
   -f {compoundOutputFileLuid0}.csv \
   -l {compoundOutputFileLuid1} \
   -sep COMMA \
   -b ', False,' \
```

```
   -h 1 \
   -c LIMSID \
   -pre 'Sample '"
```

LIMS v4.0 or later:

```
bash -c "/opt/gls/clarity/bin/java -cp /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar driver_file_generator \
   -i {processURI:v2:http} \
   -u {username} \
   -p {password} \ -t /opt/gls/clarity/extensions/ngs-
   common/v5/EPP/conf/readonly/bioA_driver_file_template.csv \
   -o {compoundOutputFileLuid0}.csv \
   -l {compoundOutputFile1} \
&& /opt/gls/clarity/bin/java
   -cp /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar
   addBlankLines \ -i {stepURI:v2:http} \
   -u {username} \
   -p {password} \
   -f {compoundOutputFileLuid0}.csv \
   -l {compoundOutputFileLuid1} \
   -sep COMMA \
   -b ', False,' \
   -h 1 \
   -c LIMSID \
   -pre 'Sample '"
```

Example 2

These examples show the configuration of an automation trigger that runs the addBlankLines script by itself, on a local file:

LIMS v3.5 or earlier:

```
bash -c "/usr/bin/java -cp /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-
extensions.jar addBlankLines \
   -i {stepURI:v2:http} \
   -u {username} \
   -p {password} \
   -f /opt/gls/clarity/customextensions/roboticsfiles/example.csv \
   -l /opt/gls/clarity/customextensions/roboticsfiles/log.html \
   -sep COMMA \
   -b ', False,' \
```

```
    -h 1 \
    -c LIMSID"
```

LIMS v4.0 or later:

```
bash -c "/opt/gls/clarity/bin/java -cp /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar addBlankLines \
    -i {stepURI:v2:http} \
    -u {username} \
    -p {password} \
    -f /opt/gls/clarity/customextensions/roboticsfiles/example.csv \
    -l /opt/gls/clarity/customextensions/roboticsfiles/log.html \
    -sep COMMA \
    -b ', False,' \
    -h 1 \ -c LIMSID"
```

Convert CSV to Excel

Compatibility: BaseSpace Clarity LIMS v3.1.0 or later; NGS Extensions Package v5.4.0 or later

Included as part of the NGS Extensions package, the convertToExcel script is designed to convert separated-value files (eg CSV) to Microsoft Excel spreadsheets of type XLS and XLSX.

- The script can be run on comma- and tab-separated files with any file extension. The original file is not edited, unless its name matches the name given for the output file.
- The script can update an existing Excel spreadsheet or produce an entirely new one.
- When updating an existing Excel spreadsheet, if this spreadsheet does not have a file extension XLSX will be used by default.
- A single worksheet is updated with the input file contents. When producing a new Excel spreadsheet, this worksheet name may optionally be specified. Otherwise, The default name will be used.
- The worksheet name must be provided when updating an existing Excel spreadsheet. If the worksheet exists, its contents will be overwritten with the contents from the input file. Otherwise, a new worksheet will be added.
- Each line in the input file becomes a row in the output file, and its values are placed into the cells of that row. The first value in the input file becomes the value of cell A:1, and so forth.
- When updating an existing worksheet, cells that are not overwritten by values from the input file are left untouched. For example, there may be a footer section that is not updated.
- The Excel file produced may be written to a location accessible from the LIMS server (a location on the server, a mounted drive, or a shared file store for example) and also attached in the LIMS via quick Attach. If both options are specified, the script will warn if the file cannot be written and report an error if the file cannot be uploaded.

- The cell types currently supported are Numeric, Boolean, Blank, and String.
- Supported number formats include period (.) as the decimal point and numbers that include an exponential (eg, 1e-8 or 4E2).
- Boolean values are case-insensitive.

Macros and equations are not supported when updating an existing Excel file. Other cells in the file that depend on the new values will not be updated when the worksheet is.

Script Parameters and Usage

The convertToExcel script uses the following parameters:

-u	LIMS username (Required)	
-p	LIMS password (Required)	
-i	LIMS step URI (Required)	
-logFileLIMSID	LIMSID of the log file that will be generated (Required)	
-logFileName	Custom name to be given to the generated log file (Optional) The name must begin with 'logFileLIMSID', or else the file will not be attached to the LIMS.	• If the name does not end in '.html', then '-LogFile.html' will be appended to the end of the file name. • Default is ${logFileLIMSID}-LogFile.html.
-srcLIMSID	The LIMSID of the file to be converted (Required if not providing inputFileName)	• If no local file is found in the current directory whose name starts with srcLIMSID, an attempt will be made to download the file from the LIMS. • You may only provide one of either inputFileName or srcLIMSID.
-inputFileName	Name of the input file to be converted (Required if not providing srcLIMSID)	• Assumes that the file is accessible from the LIMS server (either local to the server or available in a mounted drive or shared file store). • You may only provide one of either inputFileName or srcLIMSID.

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-destLIMSID	The LIMSID of the result file to attach the output XLS/XLSX file to. (Required if not providing outputFileName, or if using quickAttach.)	• At least one of outputFileName and destLIMSID are required.
-outputFileName	Name to be given to the output XLS/XLSX file. (Required if not providing destLIMSID.)	• Default name is {destLIMSID}.xls(x), based off the file type being created. • At least one of outputFileName and destLIMSID are required.
-q quickAttach	Typically used when the EPP invocation calls a subsequent script that must access the file via the API. (Optional)	• If true, destLIMSID is required and the following occurs: – When the script has completed running, it will attach the file to the LIMS via FTP, rather than writing it to disc to be attached by AI (see note for information about file names and AI). • Default is false.
-s separator	The separator used in the input file. (Optional)	• Options are COMMA or TAB. • Default is COMMA.
-xls generateXLS	Determines the output file type and extension. XLS and XLSX are supported. (Optional)	• If true, then the input file will be converted to an XLS file. • If false, then the input file will be converted to an XLSX file. • Default is false. • This parameter is ignored if updateFileName or updateFileLIMSID are provided. The file provided to update will be used to determine the output file type, and if not available XLSX will be used by default.
-worksheet worksheetName	Name of the worksheet to create or update. Must be provided when updating an existing file. (Optional)	• The worksheet name must be provided when either updateFileName or updateFileLIMSID are provided. • The worksheet name must not exceed 32 characters and must not contain any of the following: ? [ ] / \ : *

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-updateFileName	Name of an existing Excel file to update. (Optional)	• Assumes that the file is accessible from the LIMS server (either local to the server or available in a mounted drive or shared file store). • You may only provide one of either updateFileName or updateFileLIMSID .
-updateFileLIMSID	LIMSID of an existing Excel file to update. (Optional)	• If no local file is found in the current directory whose name starts with updateFileLIMSID, an attempt will be made to download the file from the LIMS. • You may only provide one of either updateFileName or updateFileLIMSID.

Automated Informatics (AI) will only pick up and attach files in BaseSpace Clarity LIMS if the outputFileName begins with the LIMSID of a file placeholder that exists in the LIMS.

For more information on AI, refer to the topics in the Automated Informatics forum.

The quickAttach option makes use of FTP to upload the file to the result file placeholder in the LIMS. This means that this script may not be run using this option on remote AI nodes, as it requires access to the LIMS database to retrieve the FTP credentials.

Configuration

- The convertToExcel script can be run on any step, provided there is a way to supply it with an input file to convert.
- The recommended configuration is to use a minimum of two shared result files on the step: One result file used to attach the final converted file; the other the log file. The input file placeholder may be the same as the final file destination, if the input file is to be overwritten with the script results, and likewise for an existing Excel file to be updated.
- Configure an automation trigger, usually on the Record Details view, to use the script. The input file may be attached manually or produced automatically by another script such as the sample sheet generator.
- To configure the script to both attach its output file to a placeholder in the LIMS and to write it to a location on the server (or in a directory with shared access), provide both outputFileName and destLIMSID with quickAttach. Include the destination path in outputFileName, for example -outputFileName '/opt/gls/clarity/customextensions/example/output.xls'.

Example Script Automation Strings

The following examples include various options for file handling. These options exist to reduce the FTP/Automated Informatics (AI) overhead so that the script executes faster.

For example, if quickAttach is set to true, the script will attach the file directly to the LIMS through FTP. It will only write the file locally if upload/attachment via the API fails.

Example 1: Typical Use

LIMS v3.5 or earlier:

```
bash -c "/usr/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar script:convertToExcel \
    -i {stepURI:v2:http} \
    -u {username} \
    -p {password} \
    -srcLIMSID {compoundOutputFileLuid0} \
    -outputFileName {compoundOutputFileLuid0}-converted.xlsx \
    -logFileLIMSID {compoundOutputFileLuid1}"
```

LIMS v4.0 or later:

```
bash -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar script:convertToExcel \
    -i {stepURI:v2:http} \
    -u {username} \
    -p {password} \
    -srcLIMSID {compoundOutputFileLuid0} \
    -outputFileName {compoundOutputFileLuid0}-converted.xlsx \
    -logFileLIMSID {compoundOutputFileLuid1}"
```

In this example:

1. The file currently attached in the LIMS with LIMS ID {compoundOutputFileLuid0} is downloaded.
2. The file is converted to an XLSX file with the name {compoundOutputFileLuid0}-converted.xlsx .
    - This file is left in the current local directory for AI to attach to the LIMS automatically.
3. When attached, the file overwrites the file with LIMS ID {compoundOutputFileLuid0} that was originally downloaded.
4. Finally, the log file is uploaded to the LIMS with the name {compoundOutputFileLuid1}-LogFile.html.

Example 2: Updating an attached Excel file and both writing it to a specific location and uploading the result

LIMS v3.5 or earlier:

```
bash -c "/usr/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar script:convertToExcel \
    -i {stepURI:v2:http} \
    -u {username} \
```

```
    -p {password} \
    -logFileLIMSID {compoundOutputFileLuid2} \
    -srcLIMSID {compoundOutputFileLuid0} \
    -outputFileName '/opt/gls/clarity/customextensions/example/
    {compoundOutputFileLuid1}.xls' \
    -destLIMSID {compoundOutputFileLuid1} \
    -worksheet 'Samples' \
    -updateFileLIMSID {compoundOutputFileLuid1} \
    -q ‘ true ’”
```

LIMS v4.0 or later:

```
bash -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar script:convertToExcel \
    -i {stepURI:v2:http} \
    -u {username} \
    -p {password} \
    -logFileLIMSID {compoundOutputFileLuid2} \
    -srcLIMSID {compoundOutputFileLuid0} \
    -outputFileName '/opt/gls/clarity/customextensions/example/
    {compoundOutputFileLuid1}.xls' \
    -destLIMSID {compoundOutputFileLuid1} \
    -worksheet 'Samples' \
    -updateFileLIMSID {compoundOutputFileLuid1} \
    -q ‘ true ’”
```

In this example:

1. The input file currently attached in the LIMS with LIMS ID {compoundOutputFileLuid0} is downloaded.
2. The Excel file to update, currently attached in the LIMS with LIMS ID {compoundOutputFileLuid1} , is downloaded.
3. The file to update has a worksheet with the name Samples updated (or overwritten, if already present in the file) using the contents of the input file.
4. The resulting file is written to /opt/gls/clarity/customextensions/example as {compoundOutputFileLuid1}.xls.
5. Because quickAttach is passed as true:
    - The file is added to the LIMS directly with FTP with the LIMS ID {compoundOutputFileLuid1}.
    - This overwrites the Excel file to update, which was previously attached here.
6. Finally, the log file is uploaded to the LIMS with the name {compoundOutputFileLuid2}-LogFile.html.

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Example 3: Use with sample sheet generator

LIMS v3.5 or earlier:

```
bash -c "/usr/bin/java -cp /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar driver_file_generator \
   -i {processURI:v2:http} \
   -u {username} \
   -p {password} \
   -t /opt/gls/clarity/extensions/ngs-common/v5/EPP/conf/readonly/bioA_driver_
   file_template.csv \
   -o {compoundOutputFileLuid0}.csv \
   -l {compoundOutputFileLuid1}-LogFile.html \
&& /usr/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-
extensions.jar script:convertToExcel \
   -i {stepURI:v2:http} \
   -u {username} \
   -p {password} \
   -inputFileName {compoundOutputFileLuid0}.csv \
   -destLIMSID {compoundOutputFileLuid8} \
   -quickAttach true \
   -logFileLIMSID {compoundOutputFileLuid1}"
```

LIMS v4.0 or later:

```
bash -c "/opt/gls/clarity/bin/java -cp /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar driver_file_generator \
   -i {processURI:v2:http} \
   -u {username} \
   -p {password} \
   -t /opt/gls/clarity/extensions/ngs-common/v5/EPP/conf/readonly/bioA_driver_
   file_template.csv \
   -o {compoundOutputFileLuid0}.csv \
   -l {compoundOutputFileLuid1}-LogFile.html \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar script:convertToExcel \
   -i {stepURI:v2:http} \
   -u {username} \
   -p {password} \
   -inputFileName {compoundOutputFileLuid0}.csv \
   -destLIMSID {compoundOutputFileLuid8} \
   -quickAttach true \
   -logFileLIMSID {compoundOutputFileLuid1}"
```

In this example:

1. A driver file is generated with the name {compoundOutputFileLuid0}.csv, and the {compoundOutputFileLuid1}-LogFile.htm l log file is created by the sample sheet generator.
2. The conversion script is executed on {compoundOutputFileLuid0}.csv.
3. Because quickAttach is passed as true and no outputFileName was provided, after the file has been converted:
    - It is added to the LIMS directly with FTP with the LIMS ID {compoundOutputFileLuid8}.
    - No file is created locally.
4. As the input file is converted, log messages are appended to the {compoundOutputFileLuid1}-LogFile.html file.

Because the destLIMSID for the convertToExcel script differs from the LIMS ID in the name of the input CSV file, the CSV file is also uploaded by AI to the LIMS and both files will be available separately.

Example 4: Use with sample sheet generator and add blank lines

LIMS v3.5 or earlier:

```
bash -c "/usr/bin/java -cp /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar driver_file_generator \
    -i {processURI:v2:http} \
    -u {username} \
    -p {password} \
    -t /opt/gls/clarity/extensions/ngs-common/v5/EPP/conf/readonly/bioA_driver_
    file_template.csv \
    -o {compoundOutputFileLuid0}.csv \
    -l {compoundOutputFileLuid1}-LogFile.html \
&& /usr/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-
extensions.jar \
    -i {stepURI:v2:http} \
    -u {username} \
    -p {password} \
    script:addBlankLines \
        -f {compoundOutputFileLuid0}.csv \
        -l {compoundOutputFileLuid1}-LogFile.html \
        -sep COMMA \
        -b ', False,' \
        -h 1 \
        -c LIMSID \
        -pre 'Sample ' \
```

```
    script:convertToExcel \
        -inputFileName {compoundOutputFileLuid0}.csv \
        -destLIMSID {compoundOutputFileLuid0} \
        -quickAttach false \
        -xls true \
        -logFileLIMSID {compoundOutputFileLuid1}"
```

LIMS v4.0 or later:

```
bash -c "/opt/gls/clarity/bin/java -cp /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar driver_file_generator \
    -i {processURI:v2:http} \
    -u {username} \
    -p {password} \
    -t /opt/gls/clarity/extensions/ngs-common/v5/EPP/conf/readonly/bioA_driver_
    file_template.csv \
    -o {compoundOutputFileLuid0}.csv \
    -l {compoundOutputFileLuid1}-LogFile.html \
&& /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar \
    -i {stepURI:v2:http} \
    -u {username} \
    -p {password} \
    script:addBlankLines \
        -f {compoundOutputFileLuid0}.csv \
        -l {compoundOutputFileLuid1}-LogFile.html \
        -sep COMMA \
        -b ', False,' \
        -h 1 \
        -c LIMSID \
        -pre 'Sample ' \
    script:convertToExcel \
        -inputFileName {compoundOutputFileLuid0}.csv \
        -destLIMSID {compoundOutputFileLuid0} \
        -quickAttach false \
        -xls true \
        -logFileLIMSID {compoundOutputFileLuid1}"
```

In this example:

1. Sample sheet generator creates the base driver file with name {compoundOutputFileLuid0}.csv.
2. The add blank lines script takes that file and adds extra lines for empty wells in the container, editing the file in place.

3. Finally, the convertToExcel script is run on that result.
    - In this case, the final output is an XLS file named {compoundOutputFileLuid8}.xls.
    - Because quickAttach is false, this file is written in the current local directory and it is assumed that AI will upload it to the LIMS.
    - The sample log file is appended to by all three programs that are run, and is attached to the LIMS.

Because the destLIMSID for the convertToExcel script is the same as the LIMS ID in the name of the input CSV file, the CSV file is not uploaded by AI to the LIMS and only the final XLS file will be available in the LIMS.

Rules and constraints

- The input file separators supported are comma and tab.
- Spaces between entries in the input file are not supported (eg "Sample Name, A:1" must instead be "Sample Name,A:1")

Logging

- A short message is logged after each successful action by the script.
- Any errors that occur will be logged in the log file before the script terminates.
- Warnings will also be captured in the log file, and if any occur, a notification will be sent on script completion.
- If a local log file exists that matches the log file name configured for the script, or if a file exists in the LIMS with the associated log file LIMSID, the log messages will be appended to these files. Otherwise, a new file will be created.

Parse CSV

Compatibility:

- Clarity LIMS Product Analytics v2.0.5 and later. Recent features are marked with the NGS Extensions Package version in which they first appear.
- New in NGS 5.4: -haltOnMissingSample option and support for header section values, eg, containerName.

Data might sometimes need to be parsed from an instrument result file (CSV, TSV, or other character-separated format) into Clarity LIMS Product Analytics, for the purposes of QC.

For example, suppose that a 96 well plate is run on a Caliper GX. The instrument produces a result file, which the user imports into Clarity LIMS Product Analytics. The per-sample data are parsed and stored for a range of capabilities, such as QC threshold checking, searching, and visibility in the Clarity LIMS interface.

The parseCSV script allows for the data for each well to be parsed into fields on either derived samples or result files (measurement records) that map directly to the derived samples being measured.

If the instrument result file contains data that applies to the batch of derived samples being measured, this data are stored in fields on the step.

The Script

The parse CSV script automates parsing a separated-value file, configurable but typically comma- or tab-separated, into the LIMS.

1. Data lines in the file are matched to the corresponding sample in the LIMS using well placement information.
2. A line that references well A1 of container Plate123 will have its parsed data mapped to the sample placed in well position A:1 of container Plate123 in the LIMS.
3. Values from the file are mapped to fields (known as UDFs in the API) in Clarity LIMS based on the automation configuration for the script.

Workflow and Configuration

- Configure the step to invoke the script manually via a button in Record Details screen.
- Before pressing the button that invokes the script, upload a shared result file to be parsed.
- Configure the automation command line to match the destination fields configured in Clarity LIMS.
- Create a field for each column that will be brought into the LIMS. Field names must not contain the separator used for the automation parameter string, "::".
- When using NGS v5.0 or later, fields can be configured for the step, input samples, output samples, or output result files. Versions before this release support only output result files.
- Input result files are not supported.

Script Parameters

The following table defines the parameters used by the parseCSV script.

-u {user}	LIMS username (Required)
-p {password}	LIMS password (Required)
-i {URI}	LIMS process URI (Required)
-inputFile {result file}	Instrument result file to be parsed (Required)
-log {log file name}	Log file name (Required)
-containerName {container name}	Name of column header for container name
-wellPosition {well position}	Name of column header for well position

-sampleLocation {sample location}	Name of column header for <container_name>_<well>
-measurementUDFMap {measurement UDF map}	Mapping of CSV columns to fields in the LIMS
-partialMatchUDFMap {partial match UDF map}	Mapping of CSV columns to fields in the LIMS (partial match)
-processUDFMap {process UDF map}	Mapping of CSV headers or columns to protocol step fields in the LIMS
-headerRow {header row}	Numeric index of CSV header row, starting from one (default 1)
-separator {separator}	File separator; comma used by default if not otherwise specified (default comma)
-matchOutput {boolean}	Match by input or output placement (default false)
-setOutput {boolean}	Control which artifacts to parse information into (default true)
-relaxed {boolean}	Control whether headers are optional or mandatory (default false)
-haltOnMissingSample {boolean}	Control whether the script halts execution or warns when the Container Name and Well Position cannot be determined on any line (default true) (NGS 5.4).

Association Strategy

The association strategy describes how information in the file is mapped to samples in the LIMS.

When running this script, there are two association strategies you can implement. Which strategy you choose is determined by the contents of the file that will be parsed. Both strategies rely on sample placement information (well and container name) to perform the mapping to the LIMS.

- Strategy 1: Provide the -containerName and -wellPosition parameters to the script. Use this strategy when the well and container information are found in separate columns of the file, eg "Plate123" in column "Plate Name" and "A1" in column "Well Label"
- Strategy 2: Provide the -sampleLocation parameter to the script. Use this strategy when the placement information is all found in the same column, in the following format: <container_name>_ < well >_ <free text>, eg "Plate123_A1_control" in column "Sample ID"

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Header Section Parsing (NGS 5.4)

For the association strategy provided, if matching headers are not found in the file at the provided header index, the script will then search the lines of the file that appear prior to this index (the header section) for a match.

For example, when using association strategy 1 and providing -containerName and -wellPosition, if the file contains information for only a single container the container name may only appear one time in a header section. This may look something like this for a comma-separated file: "ContainerID, plate123". With -containerName provided as "ContainerID" the script will locate the adjacent value as the one to be used as the value of the container name for the entire file and interpret the well positions as being within this container.

Mapping Parameters

Mapping parameters (measurementUDFMap, partialMatchUDFMap, and processUDFMap) determine which information is mapped from the file to fields in the LIMS.

The structure in which to provide these parameters is as follows, where the <Header Name> is the name of the data column or header section row in the file:

```
<UDF Name>::<Header Name>
```

At least one mapping parameter must be provided to map data from the file to the LIMS. The details of how each of these parameters affects the behavior of the script is described in the Parameter details section.

File Separator

While the most common file formats are *.csv (comma-separated) and *.tsv (tab-separated), the script may be configured to use any separator.

To use a comma or tab as the separator, provide these using the -separator parameter as "comma" or "tab" as they require additional handling by the script.

Boolean Parameters

The script supports several boolean parameters. Boolean parameter values must be provided in quotes, eg "true".

Script Usage

Example 1

This example uses matching Strategy 1 for a comma-separated file and maps two columns, "Region [100–1000] Conc. (ng/ul)" and ""Region[100–1000] Size at Maximum [BP], to output resutlfile fields "Concentration" and "Size (bp)" in the LIMS, respectively:

```
bash -c "/opt/gls/clarity/bin/java
-jar \/opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar \
   -i {processURI:v2:http} \
```


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```
   -u {username} \
   -p {password} \
script:parseCSV \
   -inputFile {compoundOutputFileLuid0} \
   -log {compoundOutputFileLuid1} \
   -headerRow '1' \
   -separator 'comma' \
   -containerName 'Plate Name' \
   -wellPosition 'Well Label' \
   -measurementUDFMap 'Concentration::Region[100-1000] Conc. (ng/ul)' \
   -measurementUDFMap 'Size (bp)::Region[100-1000] Size at Maximum [BP]'"
```

Example 2

This example uses matching Strategy 2 for a tab-separated file, running in relaxed mode. It maps a column to an input sample field, using that input sample placement information, and maps a header section row to a protocol step field:

```
bash -c "/opt/gls/clarity/bin/java -jar \/opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar \
   -i {processURI:v2:http} \
   -u {username} \
   -p {password} \
script:parseCSV \
   -inputFile {compoundOutputFileLuid0} \
   -log {compoundOutputFileLuid1} \
   -headerRow '1' \
   -separator 'tab' \
   -sampleLocation 'Sample ID' \
   -measurementUDFMap 'Concentration::Region[100-1000] Conc. (ng/ul)' \
   -processUDFMap 'Run date::Run Date'
   -matchOutput 'false'
   -setOutput 'false'
   -relaxed 'true'"
```

To view an out-of-the-box example included in the NGS package, review the configuration of the NanoDrop QC protocol steps included in the Initial DNA and RNA QC protocols.

Parameter Details

measurementUDFMap

This performs a 1:1 parsing of column information from the file to individual sample fields in the LIMS. The column names must match exactly. The exact destination (input/output sample or result file fields) is controlled through other script options.

partialMatchUDFMap

This allows customization of the column names that appear in the file by only matching on the first part of the column name, eg a partial match of "Sample" will match to a column customized to "Sample (internal ID)." Other than providing this flexibility, this parameter functions the same as measurementUDFMap.

If two columns are found that begin with the partial match provided, the script will log an error and stop execution.

processUDFMap

The process UDF option is provided to parse per-run information into protocol step fields in the LIMS. When provided, the script will search for a match in the header section and the data column headers of the file.

In the following example file:

- The first two lines (beginning with OPERATOR and WORKFLOW) represent a header section with information for the batch of derived samples.
- The third line (S_PLATE_ID) is the data section header (header row).
- The lines make up the data section, which contains data for each derived sample.

How it Works

- If there is a matching header in both the header section and column headers, the value from the header section will be used.
- If no matching header is found and the script isn't running in relaxed mode, the script will log an error and stop execution.
- When a match is found only among the column headers, validation is done to ensure all the values in that column are equal (because they will be mapped to a single destination field). If not all of the values are the same, a warning will be logged listing the distinct values and the field in the LIMS will not be updated.

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matchOutput Mode

This parameter is provided as a boolean true/false value (default is false). It toggles whether information from the file is matched to the LIMS by comparing it to the placement of the protocol step inputs or protocol step outputs.

- If set to False: The script uses the placement information of the inputs.
- If set to True: The script uses the placement information of protocol step outputs.

setOutput Mode

This parameter is provided as a boolean true/false value (default is true). It toggles whether per-sample information is mapped to fields on the protocol step inputs or outputs.

- If set to True: The script updates the protocol step outputs.
- If set to False: The script updates field information on the protocol step inputs.

Input samples, output samples, and output result files are supported. The script expects either output samples or output result files, not both.

The script will log an error and stop execution if there is more than one kind of per-input output configured for the protocol step.

relaxed Mode

This parameter is provided as a boolean true/false value (default is false) to toggle relaxed mode.

- If set to False: The script considers all provided header mappings to be mandatory headers and throws an exception if anything cannot be found in the file.
- If set to True: In relaxed mode, the script will log a warning if a header cannot be found in the file, and will continue execution.

haltOnMissingSample mode (NGS v5.4 and later)

This parameter is provided as a boolean true/false value (default is true) to toggle halt on missing sample mode.

- If set to False: The script will warn but continue execution when placement information for a line in the file cannot be determined. This mode can be used to handle, for example, ladder entries or footer sections, where the lines in the file will not contain valid sample information for the parser to use.
- If set to True: The script will log an error and stop execution when a line in the file is encountered where it cannot determine the placement information for a sample. This mode allows strict matching of all contents.

Additional Information

Other scripts you may find useful are as follows.

- Sample Input Sheet Generator
- NanoDrop Driver File Generation
- CaliperGX Driver File Generation
- Name Matching XML Parser

Name Matching XML Parser

Compatibility: BaseSpace Clarity LIMS v2.1.0 or later; NGS Extensions Package v4.3.0 or later

Often, data can be parsed from an instrument result file in XML format into BaseSpace Clarity LIMS, for the purposes of QC.

For example, perform a TapeStation instrument run. This produces an XML result file, which the user imports into the LIMS. The file includes information of interest for each sample, which should be parsed and stored for a range of capabilities, such as QC threshold checking, searching, and visibility in the LIMS interface.

The XmlSampleNameParser tool allows for sample data to be parsed into UDFs on result files (measurement records) that map directly to the derived samples being measured.

The XmlSampleNameParser tool is installed as a standalone jar file as part of the NGS Extensions Package. Currently it contains one script, parseXmlBySampleName.

Provided the result file is in XML format, this script can be used to match data in the file to samples in the LIMS using the measurement record LIMSID.

Values are mapped to UDFs in the LIMS using a configuration file that contains XPath mappings for the result file. (External resources, such as w3schools, can be used to learn more about Xpath, and many XML viewing tools will generate it automatically for elements of interest.)

The format for the data needed to make the association between the file contents and the sample in the LIMS is: LIMSID_NAME.

- The name is optional and is supported for readability. This means it may come from the input sample on which the step is being run.
- The LIMSID must come from the output result file, which is also where the parsed information will be stored in UDFs.

Typically, it is ideal to set up the instrument run with the sample and result file information, so that it will appear in the same format in the XML result file. To automate setup, you can use a tool such as the generic driver file generator.

The LIMSID_NAME can be provided to the instrument as the sample name, or as a comment or other field on the sample. The only conditions are that:

- The sample field that you want to use for the LIMSID_NAME must be passed into the file result file (eg via a driver file).
- The configuration file must be set up such that it can access this field from the correct location. (See Configuration File Format.)

Script Parameters and Usage

The parseXmlBySampleName script uses the following parameters, all of which are required:

-u {user}	LIMS username
-p {password}	LIMS password
-i {URI}	LIMS process URI
-inputFile {result file}	LIMSID of the XML file to be parsed.
-log {log file name}	Log file name
-configFile {configuration file name}	Parsing configuration file

Example

This example shows the script run on a manually imported TapeStation XML file that has been attached to the TapeStation DNA QC process.

LIMS v3.5 or earlier:

```
bash -c "/usr/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/XmlSampleNameParser.jar
   script:parseXmlBySampleName
      -i {processURI:v2:http}
      -u {username}
      -p {password}
      -inputFile {compoundOutputFileLuid0}
      -log {compoundOutputFileLuid1}.html
      -configFile
      /opt/gls/clarity/extensions/conf/tapestation/defaultTapeStationDNAConfig.g
      roovy"
```

LIMS v4.0 or later:

```
bash -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/XmlSampleNameParser.jar
   script:parseXmlBySampleName
      -i {processURI:v2:http}
```

```
-u {username}
-p {password}
-inputFile {compoundOutputFileLuid0}
-log {compoundOutputFileLuid1}.html
-configFile
/opt/gls/clarity/extensions/conf/tapestation/defaultTapeStationDNAConfig.g
roovy"
```

Configuration

The process type for the steps on which information will be tracked must be configured with the following output generation:

- 1x fixed ResultFile output per input
- 2x fixed ResultFile outputs applied to all inputs
- Shared output naming pattern example: {LIST:Instrument Result XML (required),XML Parsing Log}

This represents the minimum configuration. Additional shared output files may be added as required.

For each piece of information that will be parsed from the XML file and stored on the step outputs, configure desired UDFs on ResultFile and associate them with the per-input output result files for the process type.

Configuration File Format

The configuration file should be produced as a .groovy file and stored in the /opt/gls/clarity/customextensions directory. Its format allows for four types of entries:

- baseSampleXPath
- sampleNameXPath
- process.run.UDF."UDF name"
- process.output.UDF."UDF name"

The examples provided here use XPath for a TapeStation XML result file.

baseSampleXPath

- Provide this one time.
- This XPath indicates the list of samples and the associated sample information, relative to the root of the XML file. Specific sample information will be retrieved relative to this path.

sampleNameXPath

- Provide this one time.

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- This XPath indicates where the LIMS sample association information (LIMSID_NAME) can be found, relative to the sample list indicated by baseSampleXPath. Often this will be stored as the sample name or in a comment field for the sample.

```
// **Sample matching information**
// These two entries are required to locate and identify individual samples' information in the XML
file.baseSampleXPath = "/File[1]/Samples[1]/Sample[Observations!='Ladder']"
sampleNameXPath = "${baseSampleXPath}/Comment[1]/text()"
```

process.run.UDF."UDF name"

- May be provided multiple times.
- Indicates information that is tracked for the entire run, and not on individual samples.
- Typically, this will be XPath relative to the root of the XML file, as shown.
- The destination result file UDF name is specified as part of the entry name and must match the UDF name in the LIMS exactly.

```
// **Details that correspond to the whole run**
process.run.UDF."Conc. Units".xPath = "/File[1]/Assay[1]/Units [1]/ConcentrationUnit[1]/text()"
process.run.UDF."Molarity Units".xPath = "/File[1]/Assay[1]/Units [1]/MolarityUnit[1]/text()"
process.run.UDF."MW Units".xPath = "/File[1]/Assay[1]/Units [1]/MolecularWeightUnit[1]/text()"
```

In the example above, three values will be parsed into the LIMS from the XML file, represented on each individual measurement record (result file) output:

- Conc. Units
- Molarity Units
- MW Units

process.output.UDF."UDF name"

- May be provided multiple times.
- Indicates information that is tracked on individual samples.
- Typically, this will be XPath relative to the sample XPath (baseSampleXPath), as shown.
- The destination result file UDF name is specified as part of the entry name and must match the UDF name in the LIMS exactly.

```
// **Details that correspond to Samples**
process.output.UDF."Concentration".xPath = "${baseSampleXPath}/Concentration [1]/text()"
process.output.UDF."Region 1 Average Size - bp".xPath = "${baseSampleXPath}/Regions/Region[1]/AverageSize[1]/text()"
```


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```
process.output.UDF."Region 1 Conc.".xPath = "${baseSampleXPath}/Regions/Region
[1]/Concentration[1]/text()"
process.output.UDF."Peak 1 MW".xPath = "${baseSampleXPath}/Peaks/Peak[1]/Size
[1]/text()"
process.output.UDF."Peak 1 Conc.".xPath = "${baseSampleXPath}/Peaks/Peak
[1]/CalibratedQuantity[1]/text()"
```

In the example above, five values will be parsed into the LIMS from the XML file, represented on each individual measurement record (result file) output:

- Concentration
- Region 1 Average Size - bp
- Region 1 Conc.
- Peak 1 MW
- Peak 1 Conc.

Additional Information

Some other scripts you may find useful:

- Generic driver file generator
- CSV parser
- Bioanalyzer parser

Sample Placement Helper

Compatibility: BaseSpace Clarity LIMS v2.5 or later, and v3.0 or later

The sample placement helper tool allows for automated sample placement and validation in BaseSpace Clarity LIMS. It consists of the following scripts, each of which handles different use cases:

Container Name Validation

Clarity LIMS Product Analytics supports renaming of destination containers in a step in two ways:

- Renaming the containers on the Placement screen.
- Renaming the containers on the Record Details screen.

Often, the container name will be the barcode of the physical container (plate, chip, and so on) in the lab, which is entered into the system using a barcode scanner. However, it's possible for the barcodes to be small or close together and for the scanner to have a wide scanning range.

The purpose of this script is for validation that the same barcode has not been scanned twice during container renaming.

How it Works

The script validates the names of the destination containers in the step, and reports if any duplicates are found.

The script may be run on any step that includes sample placement, and may be run:

- Manually from the Record Details screen.- or -
- Automatically on transition at any point after placement has been done (for example, on exit from the Placement screen).

The script may be configured to do the following:

- Report either a warning or failure if duplicate container names are detected.
- Reset the destination container names in the step to the default value of the container LIMSID.

For details, see Modes.

Modes

Trigger Node

- Trigger mode must be provided when configuring the script to be triggered manually.
- This mode determines how the script reports the outcome of its validation to the user, which is a different underlying mechanism when run on-screen transition.

Error Mode

- The errMode parameter accepts values of "warn" and "fail."
- When in "warn" mode, you are still able to proceed in the step if duplicate destination container names are detected.
- If set to "fail," you will not be able to continue as long as duplicates are present.

Reset Mode

Typically, a duplicate name is caused by an accidental repeated scan of a barcode.

- Reset mode may be used to automatically reset the destination container names in the step if duplicates are detected.
- This option resets all destination container names in the step to each LIMSID, which is the default value used for container names in the LIMS.

Script Parameters and Usage

The script uses the following parameters:

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-i {URI}	Step URI (Required)
-u {username}	LIMS username (Required)
-p {password}	LIMS password (Required)
-t, -triggerMode	Provide as "true" if script will be triggered manually (Optional)
-errMode	Provide as "warn" or "fail" to determine behavior when duplicate container names are detected (Required)
-reset	Provide as "true" to reset all container names to their default LIMSID when duplicates are found (Optional)

Example 1: Validate container names on Record Details

In this example, the script is triggered manually on the Record Details screen. It will warn the user if duplicates are detected, and the destination container names will not be changed.

LIMS v3.5 or earlier:

```
bash -c "/usr/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/PlacementHelper.jar script:validate_container_names \ -i
{stepURI:v2:http} \ -u {username} \ -p {password} \ -errMode 'warn' \ -t 'true'"
```

LIMS v4.0 or later:

```
bash -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/PlacementHelper.jar script:validate_container_names \ -i
{stepURI:v2:http} \ -u {username} \ -p {password} \ -errMode 'warn' \ -t 'true'"
```

Example 2: Validate container Names on Transition

In this example, the script is configured to run automatically on-screen transition. It will fail if duplicates are detected, preventing the user from advancing in the step, and the destination container names will be reset to the container LIMSID.

LIMS v3.5 or earlier:

```
bash -c "/usr/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/PlacementHelper.jar
   script:validate_container_names \
      -i {stepURI:v2:http} \
      -u {username} \
      -p {password} \
      -errMode 'fail' \
      -reset 'true'"
```

LIMS v4.0 or later:

```
bash -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/PlacementHelper.jar
   script:validate_container_names \
      -i {stepURI:v2:http} \
      -u {username} \
      -p {password} \
      -errMode 'fail' \
      -reset 'true'"
```

Placement by Pattern File

Background

Some instruments or workflows require specific placement patterns based on the container type. A BaseSpace Clarity LIMS may need to place samples in an unusual pattern, such as every second well in a plate. To handle this, the ability to specify new patterns based on specific types of containers is required.

Purpose

The purpose of the Placement by Pattern File script is to automate sample placement according to a specific pattern based on container type. The script reads the pattern in from a file, allowing for easy customization.

There are two script modes:

- Source sample index placement: This is the default mode. This mode assigns an index to each input sample and their replicates, and then transfers them to a particular well in the output container. This mode allows for cherry picking of samples.
- Source well placement: This mode uses the well position of an input sample to transfer it to a certain well position in the output container. In this mode, samples from one well will always be transferred to the same well in the output container.

How it Works

Pattern files are stored in a directory that is passed as a parameter to the script.

Files are automatically selected based on the mode of the script and the container names:

- Source sample index placement is toggled with the useIndexMode parameter. When using this mode, the file name must contain "IndexedTransfer" and the selected container type.
- When using source well placement, the file name must contain the input container and selected container types of the protocol step.

For example, suppose that there are samples in a 96 well plate that are to be transferred into several 12x1 BeadChips...

- If source well placement is used, the pattern file would be named 96 well plate_12x1 HD BeadChip.tsv.
- If source sample index placement is used, the pattern file would be named IndexedTransfer_12x1 HD BeadChip.tsv (for information on the format of the pattern files, see the Pattern File Format section).

Both script modes support multiple input containers of the same type and multiple output containers of the same type. Only source sample index placement supports multiple types of input containers.

All input samples are sorted by container LIMS ID to ensure accurate placement. If useIndexMode is set to "true", samples will be further sorted to assign index. After being sorted by container LIMS ID, they are sorted based input sample well position. The sortOrder parameter specifies whether indexed samples are sorted by row or by column with respect to well position. The sortOrder parameter is only used with source index placement. Input samples are indexed from 1, and sample replicates of a particular sample are all assigned the same index.

Output containers are created and given temporary names of "Plate 1," "Plate 2," "Plate 3"... These can then be updated as desired, for example, by scanning in the barcode of the container. This temporary naming is done for ease-of-use, to make sure that the visual order in the interface is correct, making it easier to confirm that the placement pattern was followed.

> The script is intended to be used with fresh destination plates, rather than plates that exist in the system. If a preexisting container is selected, it too will have the temporary naming applied and will become "Plate 1."

It is possible to configure the script to fail or produce a warning message if the number of samples does not match the contents of the pattern file. There are three parameters to enforce the number of samples and replicates in the step: replicatesMode, minSamplesMode, and maxSamplesMode.

For example, if replicatesMode is set to "warn" and maxSamplesMode is set to "fail", then the script will produce a warning after placement occurs if the number of replicates is inaccurate and it will fail if there are more samples in the step than specified in the pattern file.

Process and Workflow Configuration

The script may be configured on any process that includes sample placement:

- Configure this script to run automatically on entry to the Sample Placement screen.
- Before using the script, confirm that the desired pattern files are correctly configured and are located in the correct location with the appropriate parameter provided. (See Script parameters and usage.)

Script Parameters and Usage

The script uses the following parameters:

-i {step URI}	Protocol step URI (Required)
-u {username}	LIMS username (Required)

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-i {step URI}	Protocol step URI (Required)
-p {password}	LIMS password (Required)
-d {pattern file directory}	Directory for placement pattern files (Required)
-useIndexMode {use index mode}	Determines whether source sample index placement is used instead of source well placement (Optional)
-replicatesMode {replicates mode}	Provide as "warn"/"fail" to determine script behavior if the number of replicates does not match the pattern file (Optional)
-minSamplesMode {min samples mode}	Provide as "warn"/"fail" to determine script behavior if the number of samples in the step is lower than specified by the pattern file (Optional)
-maxSamplesMode {max samples mode}	Provide as "warn"/"fail" to determine script behavior if the number of samples in the step is higher than specified by the pattern file (Optional)
-sortOrder {sort order}	Provide as "horizontal"/"vertical" to sort samples by row/column for source sample index placement - default is "horizontal" (Optional)

Example 1

This example uses source well placement and a pattern file placed in:

/opt/gls/clarity/extensions/conf/infinium/placementpatterns

LIMS v3.5 or earlier:

```
bash -l -c "/usr/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/PlacementHelper.jar
   script:place_samples \
      -i {stepURI:v2:http} \
      -u {username} \
      -p {password} \
      -d /opt/gls/clarity/extensions/conf/infinium/placementpatterns"
```

LIMS v4.0 or later:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/PlacementHelper.jar
   script:place_samples \
      -i {stepURI:v2:http} \
      -u {username} \
      -p {password} \
      -d /opt/gls/clarity/extensions/conf/infinium/placementpatterns"
```

Example 2

This example uses source sample index placement. Replicates mode fails the script if there is an inaccurate number of replicates, minSamplesMode warns the user that there are fewer samples than specified in the pattern file, and maxSamplesMode fails the script if there are more samples than specified in the pattern file. The pattern file is located in:

/opt/gls/clarity/extensions/quantstudio/conf/placementpatterns

LIMS v3.5 or earlier:

```
bash -l -c "/usr/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/PlacementHelper.jar
    script:place_samples \
       -i {stepURI:v2:http} \
       -u {username} \
       -p {password} \
       -d /opt/gls/clarity/extensions/quantstudio/conf/placementpatterns \
       -useIndexMode true \
       -replicatesMode fail \
       -minSamplesMode warn \
       -maxSamplesMode fail"
```

LIMS v4.0 or later:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/PlacementHelper.jar script:place_samples \
    -i {stepURI:v2:http} \
    -u {username} \
    -p {password} \
    -d /opt/gls/clarity/extensions/quantstudio/conf/placementpatterns \
    -useIndexMode true \
    -replicatesMode fail \
    -minSamplesMode warn \
    -maxSamplesMode fail"
```

Pattern File Selection

The location of the pattern file is customized using the appropriate parameter, as described in Script parameters and usage.

When source well placement is used, the exact file to use is selected based on the names of the input container type and destination container type:

- The name must contain an exact match for each of the container types (eg "384 well plate_96 well plate.tsv")
- Container type names must be separated from other text by underscores; any other text in the name is ignored. For example, "{type}_{type}.tsv" and "{type}_{type}_transfer file.tsv" are both valid pattern file names.

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- For a transfer from one container to another of the same type, "{type}.tsv" is a valid pattern file name. However, the script currently uses the first file it finds with a name containing the source and destination container types. If both "{type1}.tsv" and "{type1}_{type2}.tsv" are found in the same location, the script may not accurately detect which should be used for a protocol step that has input and output containers of type "type1." This can be avoided by using the long form for naming, ie {type1}_{type1}.tsv.

When source sample index placement is used, the pattern file name is slightly different:

- The name must contain "IndexedTransfer" along with an exact match for the destination container type (eg "IndexedTransfer_96 well plate.tsv")
- As with source well placement, container type names must be separated from other text by underscores; any other text in the name is ignored.

Pattern File Format

The pattern file format is a tab-separated file (.tsv) that has three columns with headers. These headers depend on the script mode. For source well placement, SRC_WELL, DEST_CONTAINER_INDEX, and DEST_WELL are the required headers. When useIndexMode is enabled, the pattern file must contain SRC_SAMPLE_INDEX, DEST_CONTAINER_INDEX, and DEST_WELL.

The table describes the four columns:

Column header	Description	Required for source well placement	Required for source sample index placement
SRC_WELL	Well position of the input sample, in the format A:1	Yes	No
SRC_SAMPLE_INDEX	Index of the input sample, indexed from 1	No	Yes
DEST_CONTAINER_INDEX	Output container index, indexed from 1	Yes	Yes
DEST_WELL	Destination well position of the sample, in the format A:1	Yes	Yes

Part of an example pattern file for source well placement:

An example of a pattern file for placement via source sample index. It expects samples to have eight replicates each.

Placement by Robot Transfer File

Some labs require support for a robot-driven sample placement scenario, in which sample placement is performed on the robot and is then recorded in the LIMS, without requiring manual entry of the sample placement.

For Research Use Only. Not for use in diagnostic procedures.

Purpose

The purpose of the Placement by Transfer File script is to automate sample placement according to a transfer file produced by a robot. The script covers a one-to-one, many-to-one (pooling), or one-to-many (replicates) mapping of samples for placement. Support for reagents is planned for a future release.

Available

Available as of BaseSpace Clarity LIMS v3.0. Replicate support added in NGS Extensions Package v5.3.1 (Placement Helper v2.1.0). Pooling support added in NGS Extensions Package v5.4.0 (Placement Helper v2.2.0) and requires LIMS v3.1.

How it Works

The robot produces a transfer file (worksheet, work list file, and so on) that contains information about which samples were used and their source and destination locations. Upload this file to the protocol Step Setup screen in the LIMS.

The Placement by Transfer File script automatically performs the same placement as the robot, recording the work that has been done in the lab, not manually enter this information.

The script looks at the protocol step inputs and matches these to the source information in the transfer file, then uses the destination information to place the protocol step outputs. On pooling steps, the script will have the intermediary step of creating the pools. After the pools are created, you are given the opportunity to make sure that their contents before the script does placements for the pools.

(For information on the format of the pattern files, see theTransfer File Format section.)

The script will first search for containers that exist in the LIMS with names matching the destination container names provided in the transfer file, and if only a single container matches a given destination container name it will be used. Otherwise the containers will be created using the destination container type, or selected container type if type information is not provided in the transfer file. The only case where the script will proceed if it finds multiple containers in the LIMS matching a destination container name in the transfer file is if one of those containers is the selected container for the protocol step.

The sample name column is used for validation. If the sample found in the LIMS that matches the input placement information does not have the same name, an exception will be thrown by the script.

Process and Workflow Configuration

- The script may be configured on any protocol step that includes sample placement.
- Configure this script to run automatically on entry to the Sample Placement screen. (See Script Parameters and Usage.)
- If you are pooling samples, the script must also be configured to run on entry to the Pool Samples screen. The configuration strings would be the same for both automatic EPP triggers.
- The Step Setup screen should also be enabled in the protocol step configuration, to allow for attachment of the transfer file.

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Script Parameters and Usage

The script uses the following parameters:

-i {URI}	LIMS process URI (Required)
-u {username}	LIMS username (Required)
-p {password}	LIMS password (Required)
-f {placement file LIMSID}	LIMS ID of the file used to map samples to new placements (Required)
-headerIndex {header index}	Numeric index of file header row, starting from 1(default is 1) (Optional)
-srcContainer {source container}	Name of column containing source container ID (Required)
-srcWell {source well}	Name of column containing source container well (Required)
-sampleName {sample name}	Name of column containing sample ID (name) (Optional)
-destContainer {destination container}	Name of column containing destination container ID (Required)
-destWell {destination well}	Name of column containing destination container well (Required)
-destType {destination type}	Name of column containing destination container type (Optional)
-separator {separator}	File separator, provide tab as "tab" and comma as "comma" (default is comma) (Optional)

Example 1

This example uses the minimum configuration for the script:

LIMS v3.5 or earlier:

```
bash -l -c "/usr/bin/java -cp /opt/gls/clarity/extensions/ngs-
common/v5/EPP/PlacementHelper.jar place_samples_by_robot_file
    -i {stepURI:v2:http}
    -u {username}
    -p {password}
    -f {compoundOutputFileLuid0}
    -srcContainer 'S_PLATE_ID'
    -srcWell 'S_PLATE_XY'
    -destContainer 'D_PLATE_ID' -destWell 'D_PLATE_XY'"
```

LIMS v4.0 or later:

```
bash -l -c "/opt/gls/clarity/bin/java -cp /opt/gls/clarity/extensions/ngs-
common/v5/EPP/PlacementHelper.jar place_samples_by_robot_file
    -i {stepURI:v2:http}
```

```
    -u {username}
    -p {password}
    -f {compoundOutputFileLuid0}
    -srcContainer 'S_PLATE_ID'
    -srcWell 'S_PLATE_XY'
    -destContainer 'D_PLATE_ID' -destWell 'D_PLATE_XY'"
```

Example 2

This example provides some of the optional parameters. The script will parse a tab-separated file that has its header on the third line, with additional validation on sample name and destination container type:

LIMS v3.5 or earlier:

```
bash -l -c "/usr/bin/java -cp /opt/gls/clarity/extensions/ngs-
common/v5/EPP/PlacementHelper.jar place_samples_by_robot_file
    -i {stepURI:v2:http}
    -u {username}
    -p {password}
    -f {compoundOutputFileLuid0}
    -headerIndex '3'
    -srcContainer 'S_PLATE_ID'
    -srcWell 'S_PLATE_XY'
    -sampleName 'SAMPLE_ID'
    -destContainer 'D_PLATE_ID'
    -destWell 'D_PLATE_XY'
    -destType 'D_PLATE_TYPE'
    -separator 'tab'"
```

LIMS v4.0 or later:

```
bash -l -c "/opt/gls/clarity/bin/java -cp /opt/gls/clarity/extensions/ngs-
common/v5/EPP/PlacementHelper.jar place_samples_by_robot_file
    -i {stepURI:v2:http}
    -u {username}
    -p {password}
    -f {compoundOutputFileLuid0}
    -headerIndex '3'
    -srcContainer 'S_PLATE_ID'
    -srcWell 'S_PLATE_XY'
    -sampleName 'SAMPLE_ID'
    -destContainer 'D_PLATE_ID'
    -destWell 'D_PLATE_XY'
```

```
-destType 'D_PLATE_TYPE'
-separator 'tab'"
```

Transfer File Format

Currently the script supports comma- and tab-separated formats with a single-line header followed by data rows for the transfer information.

The minimum information required in the transfer file is a column for each of the following:

- source container
- source well location
- destination container
- destination well location

For additional validation, it is possible to specify columns to use for the following:

- sample name
- destination container type

The contents of the transfer file must correspond to the number of sample outputs per input configured for the step. In the transfer file, replicates are represented as multiple lines with the same source container and well, but different destination containers or wells. A line must appear for each expected output, including replicates, or else an exception will be thrown by the script and placement will fail.

To pool multiple inputs together, make sure that the corresponding inputs have the same destination container and well. If so, and the step is configured for pooling, when the script is triggered on entry to the pooling screen your pools will be created. If the step is not configured for pooling, then such a transfer file will cause an error to be thrown by the script.

An example from a Hamilton robot (.tsv, tab-separated file) is shown:

S_PLATE_ID	SAMPLE_ID	S_PLATE_XY	D_PLATE_ID	D_PLATE_XY	280_260	CONC	USER	DATE_TIME	ERROR
BC123456	barcode01	A1	BC01	A1	1	0	TestUser	2013/02/27 14:56	No Error
BC123456	barcode02	A2	BC01	A2	3	0.1	TestUser	2013/02/27 14:56	No Error
BC123456	barcode03	A3	BC01	A3	1	0	TestUser	2013/02/27 14:56	No Error
BC123456	barcode04	A4	BC01	A4	-0.14	0	TestUser	2013/02/27 14:56	No Error
BC123456	barcode05	A5	BC01	A5	2.33	0.1	TestUser	2013/02/27 14:56	No Error
BC123456	barcode06	A6	BC01	A6	0.65	-0.1	TestUser	2013/02/27 14:56	No Error
BC123456	barcode07	A7	BC01	A7	1	0	TestUser	2013/02/27 14:56	No Error
BC123456	barcode08	A8	BC01	A8	3	0.1	TestUser	2013/02/27 14:56	No Error
BC123456	barcode09	A9	BC01	A9	1	0	TestUser	2013/02/27 14:56	No Error
BC123456	barcode10	A10	BC01	A10	-0.14	0	TestUser	2013/02/27 14:56	No Error
BC123456	barcode11	A11	BC01	A11	2.33	0.1	TestUser	2013/02/27 14:56	No Error
BC123456	barcode12	A12	BC01	A12	0.65	-0.1	TestUser	2013/02/27 14:56	No Error
BC123456	barcode13	B1	BC01	B1	1	0	TestUser	2013/02/27 14:56	No Error
BC123456	barcode14	B2	BC01	B2	3	0.1	TestUser	2013/02/27 14:56	No Error

Constraints

- Placements separated by a colon (eg, 1:1) or that are alphanumeric (eg A1) are valid. Placements that are numeric only (eg 11) are not supported.

- If the destination container type information is not supplied, the selected container type for the protocol step will be used to place the samples.

Additional Information

Some other scripts you may find useful:

- Automating container transfer based on an input file
- Parse CSV

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Packages and Reference

Additional reference information about the Illumina integrations.

Illumina Instrument Sample Sheets (NGS v5.17 & later)

Compatibility:

- Clarity LIMS v4.2 & later, with NGS Extensions v5.17 and later
- Clarity LIMS v5.1 & later, with NGS Extensions v5.17 and later

Use the Template File Generator tool to generate custom sample sheets for upload to Illumina instruments.

The driver_file_generator script is invoked in an automation command line, typically configured on the step prior to the instrument run. By default, the automation is triggered by a button on the Record Details screen.

For details on the parameters used by the script, template file contents, and how to create custom template files, see the Template File Generator documentation in the Clarity LIMS Lab Instrument Toolkit section.

MiSeq Control Software and bcl2fastq2-compatible sample sheet template files are available from the Clarity LIMS Support team.

MiSeq Sample Sheet Generation

This section summarizes the default configuration for MiSeq sample sheet generation.

Automation name	Enabled on master step	Triggered by
Generate MiSeq SampleSheet	Denature and Dilute (MiSeq v1.0)	Manual button on Record Details screen

Automation command line:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {processURI:v2} -u {username} -p
{password} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/MiSeqSamplesheet.csv \
-o {compoundOutputFileLuid1}.csv \
-q true \
-destLIMSID {compoundOutputFileLuid1} \
-l {compoundOutputFileLuid2}"
```

By default, the automation is triggered by a manual button on the Record Details screen.

bcl2fastq2 Sample Sheet Generation

This section summarizes the default configuration for bcl2fastq2 sample sheet generation.

Two bcl2fastq2 software-compatible template files are available from the the Clarity LIMS Support team.

If you would like reverse complement sequences to be included in your sample sheet, use the BCL2FASTQ_Reverse_Complement_Samplesheet.csv template.

The bcl2fastq2 sample sheet may be used with multiple instruments. The default configuration for the Illumina NextSeq integration is shown below. For information on Illumina instrument <--> bcl2fastq version compatibility, see the Illumina Supported Software Matrix.

Automation name	Configured on master step	Triggered by
Generate bcl2fastq2 NextSeq Samplesheet	Denature and Dilute NextSeq 5500/550 v1.0)	Manual button on Record Details screen

Automation command line:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/DriverFileGenerator.jar -i {processURI:v2} -u {username} -p {password} \
script:driver_file_generator \
-t /opt/gls/clarity/extensions/conf/driverfiletemplates/{udf:Samplesheet Template} \
-o {compoundOutputFileLuid1}.csv \
-q true \
-destLIMSID {compoundOutputFileLuid1} \
-l {compoundOutputFileLuid2}"
```

By default, the automation is triggered by a manual button on the Record Details screen.

To generate a custom sample sheet, complete the following steps:

LIMS v5.1 and later

1. Obtain the desired template file from the Support team.
2. Customize the template file as required. (See Template File Generator documentation in the Clarity LIMS Lab Instrument Toolkit section.)
3. Upload the customized template file to the appropriate step automation. A token for the template file is automatically added to the Tokens list.
4. In the automation command line, provide the file token as the -t (-templatePath) parameter value:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/DriverFileGenerator.jar -i {processURI:v2} -u {username} -p {password} \
script:driver_file_generator \
```

```
-t {file:myTemplate.csv} \
 -o {compoundOutputFileLuid1}.csv \
-q true \
-destLIMSID {compoundOutputFileLuid1} \
-l {compoundOutputFileLuid2}"
```

At run time, the token is replaced with the absolute path of the template file.

LIMS v4.2 and later

1. Obtain the desired template file from the Support team.
2. Customize the template file as required. (See Template File Generator documentation in the Clarity LIMS Lab Instrument Toolkit section.)
3. Upload the customized template file to the customextensions directory:

```
/opt/gls/clarity/customextensions
```

4. In the Operations Interface, on the process type External Programs tab, modify the command line so that the -t (-templatePath) parameter points to your customized template. For example:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar -i {processURI:v2} -u {username} -p
{password} \
script:driver_file_generator \
-t /opt/gls/clarity/customextensions/myTemplate.csv \
-o {compoundOutputFileLuid1}.csv \
-q true \
-destLIMSID {compoundOutputFileLuid1} \
-l {compoundOutputFileLuid2}"
```

Preconfigured Workflows Package

The Illumina Preconfigured Workflows Package (PCWP) supports the integration of Clarity LIMS with established lab protocols. The integration provides preconfigured workflows that map to lab protocols and steps, and support Illumina library prep kits, reagent kits, and controls. For detailed information on the installed workflows, see the instrument integrations and protocols documentation.

Preconfigured Workflows Package v2.0.1 Installation and Configuration

The Illumina Preconfigured Workflows Package (PCWP) v2.0.1 supports the integration of Clarity LIMS with established lab protocols.

The integration provides preconfigured workflows that map to lab protocols and steps, and support Illumina library prep kits, reagent kits, and controls. This guide provides instructions for installing the Preconfigured Workflows Package, and describes the components installed in the default configuration. For information on the installed workflows, see the instrument integrations and protocols documentation.

This version is only compatible with Clarity LIMS v4.2, and v4.3. NGS 5.19 (or later) must be installed with PCWP v2.0.1.

Preconfigured Workflows Package Contents

The PCWP is distributed in the RPM package, BaseSpaceLIMS-Pre-configured-Workflows-Package. The RPM installs the following:

- LIMS configuration. The installer allows a choice of workflows to install, and only the choices compatible with the installed LIMS version are presented.
- Database properties to configure the sequencing service.
- Templates for sample sheet generation:
  - BCL2FASTQ_Samplesheet.csv
  - BCL2FASTQ_Reverse_Complement_Samplesheet.csv
  - NovaSeq_BCL2FASTQ_Samplesheet.csv
  - NovaSeq_BCL2FASTQ_Reverse_Complement_Samplesheet.csv
  - MiSeqSamplesheetv2.csv
- Templates for sample placement:
  - 96 well plate_96 well plate.tsv
  - 1x8 Strip Tube_1x8 Strip Tube.tsv
- Templates for calculation:
  - NovaSeq_Standard_Bulk_Pool1.csv
  - NovaSeq_Standard_Bulk_Pool2.csv
  - NovaSeq_Standard_Bulk_Pool3.csv
  - NovaSeq_Xp_Bulk_Pool.csv
  - NovaSeq_Xp_Bulk_Pool2.csv
  - NovaSeq_Xp_Working_Pool.csv
  - NovaSeq_Xp_Working_Pool2.csv
- The configuration slices included in the PCWP are listed in the following section. Except for the Illumina_Instruments slices, all other configuration depends on the QC_Protocols base configuration slice.
- Illumina_Instruments
  - MiSeq
  - NextSeq
  - NovaSeq

    - LibraryPrepValidation
- QC_Protocols
    - base (the workflows depend on this configuration)
- Tier_1_Workflows
    - Nextera DNA Flex for Blood Samples
    - Nextera DNA Flex for Large Complex Genomes
    - Nextera DNA Flex for Saliva Samples
    - Nextera DNA Flex for Small Genomes
    - Nextera Rapid Capture Custom Enrichment (NRCC)
    - Nextera XT DNA
    - TruSeq Methyl Capture EPIC
    - TruSeq RNA Access
    - TruSeq Stranded mRNA
    - TruSeq Stranded Total RNA
    - TruSeq Targeted RNA Expression (TREX)
    - TruSight Tumor 170
- Tier_2_Workflows
    - AgilentOneColor5
    - AmpliSeqCancer
    - HaloplexTargetEnrichment
    - IonSmallRNA
    - IonWholeTranscriptome
    - KAPA
    - Nanopore
    - NanostringCounterGeneExpression5
    - NimblegenExome
    - PacBio-ATailing
    - PacBio
    - Raindance
    - Roche480
    - SequenomMassArray5
    - SureSelectXT2

  - SureSelectXT
  - TargetSeqLarge
  - TargetSeqSmall
  - ViiA7
- Tier_3_Workflows
  - Other

Illumina Instruments

The Illumina_Instruments MiSeq®, NextSeq, and NovaSeq configuration slices require installation of the NGS Extensions Package, but do not require installation of the QC_Protocols base configuration slice.

When installing the MiSeq, NextSeq®, and NovaSeq configuration slices, installation of the LibraryPrepValidation configuration slice is optional. LibraryPrepValidation installs the Library Prep Validation v1.1.1 workflow, which contains a single-step protocol that models the library prep required to produce normalized libraries ready for the MiSeq Sequencing v2.0, NextSeq Sequencing v1.0, and NovaSeq 6000 v3.1 workflows.

For details on these workflows, see the related MiSeq/NextSeq/NovaSeq integration documentation.

QC_Protocols base

The following table lists the configuration installed with the QC_Protocols base slice. This configuration is required to support the preconfigured workflows.

Components	Items installed
Workflows	None
Protocols	• DNA Initial QC 5.0 • Library Validation QC 5.0 • RNA Initial QC 5.0

Master steps	• Add Multiple Reagents • Adenylate ends & Ligate Adapters (TruSeq DNA) 5.0 • Pool Samples • Aggregate QC (DNA) 5.0 • Aggregate QC (Library Validation) 5.0 • Aggregate QC (RNA) 5.0 • Bioanalyzer QC (DNA) 5.0 • Bioanalyzer QC (RNA) 5.0 • CaliperGX QC 5.0 • NanoDrop QC (DNA) 5.0 • NanoDrop QC (RNA) 5.0 • PicoGreen QC (DNA) 5.0 • PicoGreen QC (RNA) 5.0 • Qubit QC (DNA) 5.0 • Qubit QC (RNA) 5.0 • Tapestation QC (DNA) 5.0 • Tapestation QC (RNA) 5.0 • qPCR QC 5.0
Container types	• 384 well plate • 96 well plate • Tube • Bioanalyzer DNA 1000 Chip • Bioanalyzer DNA High Sensitivity Chip • Bioanalyzer RNA Nano Chip • Bioanalyzer RNA Pico Chip
Label groups/Reagent categories	• TruSeq Custom Amplicon Adapters (A7-A5) • TruSeq DNA LT Adapters (AD series) • TruSeq Stranded mRNA LT Adapters (AR series) • TruSeq HT Adapters (D7-D5) • Nextera DNA Adapters (N7-N5, S7-S5) • Nextera Enrichment & Rapid Capture Adapters (N7-E5) • NoIndex • TruSeq Small RNA Adapters (RPI series)
Reagent kits	• TruSeq DNA PCR-Free Kit - Adapter Plate Box • TruSeq DNA PCR-Free Kit - Core Reagents Box • TruSeq DNA PCR-Free Kit - SP Beads Box

Controls	• Endogenous Positive Control • Exogenous Positive Control • No Amplification Control • No Reverse Transcriptase Control • No Template Control • PhiX v3
Sample global fields/Submitted sample UDFs	• Application • Pooling • Progress • Read Length • Reference Genome • Sample Buffer • Sample Conc. • Sample Type • Sequencing Coverage • Sequencing Method • Units • Volume (µl)
Container UDFs	• Container Name • LIMS ID (Container) • Well
Project UDFs	• Project Name
Derived sample global fields/Analyte UDFs	• A260/280 ratio • Concentration • Conc. Units • Sample Name • Waiting • Workflow

ResultFile (Measurement) UDFs	• 28s/18s ratio • A260 • A260/230 ratio • A260/280 ratio • BA Sample Name • Conc. Units • Concentration • MW Units • Molarity Units • Number of Peaks found • Number of Regions found • Peak 1% Integrated Area • Peak 1 Conc. • Peak 1 MW • Peak 1 Molarity • Peak 1 Size - bp • Peak 2% Integrated Area • Peak 2 Conc. • Peak 2 MW • Peak 2 Molarity • Peak 2 Size - bp • Peak 3% Integrated Area • Peak 3 Conc. • Peak 3 MW • Peak 3 Molarity • Peak 3 Size - bp • Peak 4% Integrated Area • Peak 4 Conc. • Peak 4 MW • Peak 4 Molarity • Peak 4 Size - bp • Peak 5% Integrated Area • Peak 5 Conc. • Peak 5 MW • Peak 5 Molarity • Peak 5 Size - bp • RIN • RNA Area • Region 1% of Total • Region 1 Average Size - bp • Region 1 Conc. • Region 1 Molarity • Region 2% of Total • Region 2 Average Size - bp • Region 2 Conc. • Region 2 Molarity • Region 3% of Total • Region 3 Average Size - bp • Region 3 Conc. • Region 3 Molarity

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Tier 1 Workflows

The Tier_1_Workflows configuration slices require installation of the NGS Extensions Package and the QC_Protocols base configuration slice. The Tier_1_Workflows configuration slices install the following protocols:

- Nextera DNA Flex for Blood Samples v1.1.1
- Nextera DNA Flex for Large Complex Genomes v1.1.1
- Nextera DNA Flex for Saliva Samples v1.1.1
- Nextera DNA Flex for Small Genomes v1.1.1
- Nextera Rapid Capture Custom Enrichment (NRCC) v1.1.1
- Nextera XT DNA v1.1.1
- TruSeq Methyl Capture EPIC v1.1.1
- TruSeq RNA Access v1.1.1
- TruSeq Stranded mRNA v1.1.1
- TruSeq Stranded Total RNA v1.1.1
- TruSeq Targeted RNA Expression (TREX) v1.1.1
- TruSight Tumor 170 v1.1.1

For details on these workflows, see the articles in the Preconfigured Workflows Package documentation section.

Tier 2 Workflows

The Tier_2_Workflows configuration slices require installation of the NGS Extensions Package and the QC_Protocols base configuration slice. The Tier_2_Workflows configuration slices install the following protocols and steps:

- Agilent One Color 5.0
- Ion AmpliSeq Cancer Panel Sample Prep 5.0
- HaloPlex Target Enrichment 5.0
- Ion Small RNA Library Prep 5.0
- Ion Whole Transcriptome Library Prep 5.0
- KAPA LTP Library Prep 5.0
- Burn--in Genomic DNA Sequencing (Oxford Nanopore) 5.0
- Nanostring nCounter Gene Expression 5.0
- Nimblegen Exome SeqCapEZ v3 5.0
- Template Prep 250 bp (PacBio) 5.0
- Template Prep 2 Kb (PacBio) 5.0

- Raindance dPCR
- Roche 480 qPCR
- Sequenom MassARRAY 5.0
- SureSelect XT2
  - SureSelect XT2 - PreCapture 5.0
  - SureSelect XT2 - Hybridization 5.0
  - SureSelect XT2 - PostCapture 5.0
- SureSelect XT
  - SureSelect XT DNA Prep 5.0
  - SureSelect XT Target Enrichment 5.0
- Ion TargetSeq Sample Prep (Large Region) 5.0
- Ion TargetSeq Sample Prep (Small Region) 5.0
- ViiA 7 - Quantitation

Tier 3 Workflows

The Tier_3_Workflows configuration slice requires installation of the NGS Extensions Package and the QC_Protocols base configuration slice. The Tier_3_Workflows configuration slice installs the following protocols (no workflows are installed):

- Initial Sample Preparation
- Immunohistochemistry
- Fluorescence in situ hybridization
- Standard Banding Karyotyping 5.0
- Spectral Karyotyping 5.0

Package Installation

If this installation is a new BaseSpace Clarity LIMS installation, install the QC_Protocols base configuration before installing the preconfigured workflows. This base configuration is included in the standard installation process. See RPM installation section for more information.

After a workflow is selected, the installer validates the import of the workflow, and provides the Warning/Error details in STDOUT, allowing proceeding to the import (or cancellation).

Provided or Installed Automatically

The PCWP RPM provides instructions for where to find the bash script that launches the PCWP configuration installer, namely:

```
/opt/gls/clarity/config/pre-configured-workflows-installer.sh
```

The PCWP RPM automatically installs template files for the Template File Generator (TFG), only accessible after a configuration that uses them is installed. The template files are installed under:

```
/opt/gls/clarity/extensions/conf/driverfiletemplates
```

The PCWP RPM automatically installs pattern files for the Sample Placement Helper, used by some configuration slices installed with PCWP. The pattern files are installed under:

```
/opt/gls/clarity/extensions/conf/placementtemplates/96_to_96_stamp
/opt/gls/clarity/extensions/conf/placementtemplates/Strip_Tubes
```

RPM Installation

To install the RPM:

1. Log in as the root user on the server for the PCWP RPM installation
2. Use the yum command to install the RPM. Include the --enablerepo command-line argument to enable the repo. The Support team provides the repo file and appropriate name.

```
yum install BaseSpaceLIMS-Pre-configured-Workflows-Package --enablerepo=<< repo name info from support >>
```

3. Type y to proceed with the RPM installation and install the package.
4. If the NGS Extensions Package is not already installed, or if a version earlier than v5.19 is installed, install/upgrade the NGS Extensions Package to v5.19 or later.

For PCWP installer commands, parameters, and options, see the following sections.

PCWP Installer Tool Parameters and Operations

Parameters

The PCWP configuration installer tool must be run as the glsjboss user. The following commands are available:

Parameter	Description	Example
--operation, -o	Run mode for the installer: [help, list, install]	`bash /opt/gls/clarity/config/pre-configured-workflows-installer.sh -o list`
--headless, -h	Run the installer in headless mode (for the install operation only).	`bash /opt/gls/clarity/config/pre-configured-workflows-installer.sh --headless -o install QC_Protocols.base`
--log, -l	Log file destination. Default value is workflows-installer.log	`bash /opt/gls/clarity/config/pre-configured-workflows-installer.sh -l workflows-installer.log`

PCWP List Operation

When run with the list operation, the PCWP installer lists all options for configuration install.

To use the list operation as the glsjboss user, run the following command to view the complete list of PCWP workflows:

```
/opt/gls/clarity/config/pre-configured-workflows-installer.sh -o list
```

The package Id is the value provided to the tool when running in install mode. See the following section for details.

PCWP Install Operation

Use the install operation to install configuration slices.

To install a specific configuration slice, run the following command. Specify both the name of the parent item and the name of the configuration slice, separated by a period:

```
/opt/gls/clarity/config/pre-configured-workflows-installer.sh -o install
<Parent_Item_name>.<slice_name>
```

For example, to install the QC_Protocols base slice:

```
opt/gls/clarity/config/pre-configured-workflows-installer.sh -o install QC_
Protocols.base
```

Using the All Option

There is a special option, all, which is available in two scenarios:

- At the top level to install every configuration package from the PCWP.
- At the parent item level, for all parent items that contain multiple configuration slices, for example, Tier_1_Workflows.

To install all package components, run the following command to install the QC_Protocols base slice first:

```
bash /opt/gls/clarity/config/pre-configured-workflows-installer.sh -o install
all
```

To install all workflows from a specific parent item, run the following command:

```
bash /opt/gls/clarity/config/pre-configured-workflows-installer.sh -o install
Parent_Item_name.slice_name
```

For example, to install all Tier 1 workflows:

```
bash /opt/gls/clarity/config/pre-configured-workflows-installer.sh -o install
Tier_1_Workflows.all
```

Because QC_Protocols and Tier_3_Workflows contain only one configuration slice, the all option is not available for these items. Specifying the all option in the command results in an error.

Using Headless Mode

Headless mode allows the install operation to complete without prompting for any input, and is most useful when automating an installation. The installation process runs through the validation phase before importing the configuration. However if a conflict is found, the package in conflict is automatically skipped.

Installation Validation

The installer validates the import of the configuration. If any conflicts are detected during validation, a warning message opens with the following options:

- f / Import anyway: This option runs the config slicer tool import command for the slice and allows the tool to handle conflicts as it normally would. It does not run config slicer in importAndOverwrite mode.
- s / Skip this workflow: Does not import the slice for which conflicts were found, but continues the import of other slices selected.
- a / Abort: Aborts all import operations. The conflicts found during validation are captured in the log files and can be reviewed.

Tracking and Logging the Installation

After installation completes, a record is stored in the database installhistory for reference.

To view the install history record, use the following command:

```
java -jar /opt/gls/clarity/tools/propertytool/omxprops-ConfigTool.jar
getInstallHistory
```

Certain upgrade scenarios, or attempts to resolve conflicts, can incorrectly cause a configuration slice to be installed more than one time. This error causes multiple entries.

The PCWP version is tracked, providing a breadcrumb to determine which version of a configuration slice was installed.

Logging

Up to 3 log files are captured when a configuration package is installed:

- workflows-installer.log
- configslicer.log
- omxprops.log

The log files are generated in the present working directory. Create and then change to the desired directory where the files are generated.

```
workflows-installer.log
```

The name and location of this log file cannot be determined using the command line options. The log file captures the output of the PCWP installer tool.

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```
configslicer.log & omxprops.log
```

These files are raw output from their respective tools. Some workflow selections in the PCWP include both a configuration slice and database properties for import.

PreConfigured Workflow Package v2.0.1 Release Notes

This is an optional update for customers interested in any instrument integration package requiring this release or in the new workflow available with this release.

Compatibility

The Preconfigured Workflow Package v2.0.1 is compatible with:

- Clarity LIMS v4.x (v4.2 and above)
- NGS Extensions Package v5.19 or later

New Features

This release provides the NovaSeq 6000 v3.1 preconfigured workflow.

Defect Repairs

- The NovaSeq workflow allows reverse complement workflow regardless of the PairedEnd option selected.
- The NovaSeq workflow displays the correct lane number in the Automated-NovaSeq Run step for runs using a NovaSeq Standard workflow.

Known Issues

None

Next Generation Sequencing (NGS) Package

The Illumina Preconfigured Workflows Package (PCWP) supports the integration of Clarity LIMS with established lab protocols. The integration provides preconfigured workflows that map to lab protocols and steps, and support Illumina library prep kits, reagent kits, and controls. For detailed information on the installed workflows, see the instrument integrations and protocols documentation.

NGS Extensions Package v5.25.0 Release Notes

These Release Notes detail the key changes to software components for the Next Generation Sequencing (NGS) Extensions Package v5.25.0.

Compatibility

Clarity LIMS v6.2 and later

New Features

Added the download_attachments script. This script copies one or more result files attached in a step to a user-specified directory within Clarity LIMS. For example, this script can copy a generated sample sheet to a mounted directory so that the user can access it directly from the instrument control

software.

Defects Fixed

None

Known Issues

An error occurs when reformatting the date using the Lab Logic Toolkit with the Date utility class. The date reformatting can be done with `java.time` instead.

Other

The `-s` in the stopwatch parameter is replaced with `-stopwatch` in NGS v5.21 and later.

NGS Extensions Package v5.24.0 Release Notes

These Release Notes detail the key changes to software components for the Next Generation Sequencing (NGS) Extensions Package v5.24.0.

Compatibility

Clarity LIMS v6.2 and later

New Features

Added the following scripts:

- `validate_selected_container`
- `validate_same_udf_valuee_for_analytes`

Defects Fixed

None

Known Issues

An error occurs when reformatting the date using the Lab Logic Toolkit with the Date utility class. The date reformatting can be done with `java.time` instead.

Other

The `-s` in the stopwatch parameter is replaced with `-stopwatch` in NGS v5.21 and later.

NGS Extensions Package v5.23.0 Release Notes

These Release Notes detail the key changes to software components for the Next Generation Sequencing (NGS) Extensions Package v5.23.0.

Compatibility

Clarity LIMS v6.2 and later

New Features

- Updates Java and third-party dependency libraries.
- Updates Groovy to v3.0.7.
- Stopwatch parameter `-s` is replaced with the `-stopwatch` parameter for NGS 5.21 and later.

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Defects Fixed

- The sample sheet for the MiSeq now shows the following information:
  - The pool name instead of the sample name when the pool contains only one sample.
  - The well and plate information for submitted samples instead of for libraries.
- Fixed issue where the template file generator created duplicate lines for the first data source entry when using multiple data sources.
- Fixed issue where the driver file generator did not append the existing log file.
- The `changeWorkflow` script on the No Output step reflects the correct number of samples being routed.
- Fixed the Insecure Input Validation (SRCCLR-SID-22742) vulnerability.
- Fixed degradation of the LLTK script performance when updating the custom field with an empty string.
- Fixed degradation of the log file download performance when automation performs write actions on an existing log file.
- Improved the LLTK script error messages to show details of artifacts.

Known Issues

- An error occurs when reformatting the date using the Lab Logic Toolkit with the Date utility class. The date reformatting can be done with `java.time` instead.

NGS Extensions Package v5.22.0 Release Notes

These release notes detail the key changes to software components for Next Generation Sequencing (NGS) Extensions Package 5.22.0.

Compatibility

Clarity LIMS v5.0 and later

New Features

Features provided with this release are as follows:

- Supports the new NovaSeqDx v1.0 workflow with a new script to validate all input samples in the Make Bulk Pool step as either RUO or Dx.
- Provides a new script to calculate adjusted per sample value for multiple pools.
- Bug fixes.

Defects Fixed

- Fixed the driver file generator not removing duplicate lines by adding new -d flag for the user to either remove or keep duplicated rows when using the `driver_file_generator` script.
- Fixed the auto-placement helper not using correct template in the directory by adding the following criteria for the placement template file name:

  - Criterion 1: The file name must contain the source container type name and the destination container type name (eg, `SOURCE CONTAINER TYPE NAME_DEST CONTAINER TYPE NAME.tsv`).
  - Criterion 2: The source container type name must be in front of the destination container type name (eg, `random string_SOURCE CONTAINER TYPE NAME_DEST CONTAINER TYPE NAME.tsv`).

Known Issues

An error occurs when reformatting the date using the Lab Logic Toolkit with the Date utility class. Date reformatting can be done using java.time instead.

NGS Extensions Package v5.21.0 Release Notes

These release notes detail the key changes to software components for the Next Generation Sequencing (NGS) Extensions Package since version 5.20.3.

Compatibility

Clarity LIMS v5.0 and later

New Features

Features provided with this release are as follows:

- Support for Clarity LIMS v6.
- Tech refresh to upgrade third-party libraries.
- Fixes to address the following Apache log4j vulnerabilities:
  - CVE-2021-44228
  - CVE-2021-45046
  - CVE-2021-45105
  - CVE-2021-44832

Defects Fixed

- The parseBioAnalyzer script now correctly parses the Conc. Units custom field.
- Log file for the parseBioAnalyzer automation is now logged when run more than once.

Known Issues

When the same container type is used as the input and output container type, the sample placement helper script (`PlacementHelper.jar script:place_samples`) looks for the first template file that contains the input container type string in the file name when scanning the specified template files location. For example, if a 96-well plate is used as the input and output container type and there are multiple template files in the same location, the script uses the first file that contains the input container type string. To avoid this issue, select a location that only has one template file.

This bug will be fixed in the upcoming NGS v5.22.0 release.

NGS Common Package v5.20.3 Release Notes

These release notes detail the key changes to software components for the NGS Common Package since version 5.20.2.

This optional update is for customers interested in new features available with this release.

Compatibility

The Clarity LIMS Next Generation Sequencing (NGS) package v5.20.3 is compatible with Clarity LIMS v4.2 through 5.x.

New Features

- This release includes a security patch to address the XML Entity Expansion Attacks issue.
- The Calculate Volumes automation script now returns individual calculation results for samples with the same name but in a different well or container.

Defect Repairs

None

Known Issues

None

NGS Common Package v5.20.2 Release Notes

These Release Notes detail the key changes to software components for the NGS Common Package since version 5.20.

This is an optional update for customers interested in new features available with this release.

NGS 5.20.1 was not released externally due to critical bugs.

Compatibility

The Clarity LIMS Next Generation Sequencing (NGS) package is compatible with Clarity LIMS v4.2 through 5.x

New Features

This release supports the security improvement made to the management of passwords in Clarity LIMS v5.4

Defect Repairs

None

Known Issues

None

Accession Reagent Kit Lots

The Clarity LIMS NGS Extensions Package includes the `AccessionReagentKitLots` script, which automates the accessioning of reagent lots based on container names. This section provides details on the script and its parameters, and LIMS configuration requirements.

Script Overview

Designed for use in automated workflows, the `AccessionReagentKitLots` creates a new reagent lot, matching the Reagent Kit Name specified in the command line to a reagent kit configured in the LIMS.

The script populates the lot details according to the following logic (see Script Parameters section for details):

- Lot Name = Lot name (specified by `lotName` parameter)
- Lot Number = Container name (specified by `from` arameter)
- Expires = now + expiry days (specifiedby `expiryDays` parameter)
- Status of Reagent Lot toggle switch is set to Active

Before accessioning the reagent lot, the script verifies that:

- The `expiryDays` parameter value is greater than 0, and is a whole number
- The reagent lot does not already exist in the LIMS - lots are not reusable.

The script checks whether there is an existing reagent lot in the LIMS that matches the combination of lot name + lot number provided. If so, validation fails. (Kit name is not checked during this validation process.)

If a unique combination of lot name + lot number is provided, the LIMS creates the new lot—even if there are already lots in the LIMS matching either the name or the number.

Note that it is therefore possible to create both of these lots:

- Lot 'ABC' with number '123'
- Lot 'ABD' with number '123'

Script Parameters

-u {username} -username {username}	(Required) LIMS login username
-p {password} -password {password}	(Required) LIMS login password
-i {stepURI:v2} -stepURI {stepURI:v2}	(Required) LIMS step URI

-log {logFileLIMSID} -logFileName {logFileLIMSID}	(Required) LIMS ID of the log file placeholder
-from <outputContainer / inputContainer>	(Required) Must be either 'outputContainer' or 'inputContainer'. This dictates where the 'Container Name' value is copied from. This is used to create the lot number. Only supports output containers on steps that output samples. See *Rules and Constraints* on page 2563.
-reagentKit <Reagent Kit Name>	(Required) Reagent Kit name on which the generated lot is based.
-lotName <Reagent Lot Name>	(Required) Reagent Lot name to assign to the lot
-expiryDays <#>	(Required) Number of days the lot's expiry date will be set to. Must be greater than zero (script checks for this).
-t <true/false> -testMode <true/false>	(Optional) Runs script in test mode. Script is triggered manually via button on Record Details screen. Defaults to false.

Command Line Example

```
bash -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2:http} -u {username} -p {password} script:accessionReagentKitLots -log {compoundOutputFileLuid0} -from 'outputContainer' -reagentKit '<Reagent Kit Name>' -lotName '<Lot Name>' -expiryDays <#>"
```

Configuration

The following sections describe the configuration intended to support the `AccessionReagentKitLots` script.

Master Step Custom Fields

Configure master step custom fields (step UDFs in v4.x) for reagent kit name and lot name, and display them in the Step Details area of the Record Details screen. When running the step, the user fills in these values and they are supplied to the `reagentKit` and `lotName` parameters in the script. In this scenario, configure the automation command line as follows:

```
script:accessionReagentKitLots -log {compoundOutputFileLuid0} -from 'outputContainer'
```


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```
-reagentKit '<udf:Reagent Kit Name>' -lotName '<udf:Lot Name>' -expiryDays <#>"
```

Automation trigger

The script is intended to be automatically triggered at the end of a step. However, you may include the -t option to run the script in test mode and trigger the script via button on the Record Details screen.

Rules and Constraints

- Reagent Kit Name specified in the command line must match a reagent kit configured in the LIMS.
- `expiryDays` parameter value must meet the following criteria:
  - Must be a whole number greater than 0.
  - Must be a numeric value (i.e., '1' not 'one').
- When specifying the `from` parameter, note that the script only supports the value 'outputContainer' if the step outputs are samples. Step output measurement records with placement are not supported.
- Accessioning reagent lots with this script sets each reagent lot status to Active until the expiry date is reached. You cannot use the script to add reagent lots with status set to Pending.
- The step should only be run with a single container matching the `from` definition.
- If `from` is defined as the output container, the step can have multiple input containers but all samples must be placed in the same output/destination container. This is because the script only looks up a single container name. If there are multiple output containers in the step, the script will not fail; however, it will choose a container arbitrarily, which could result in unexpected behavior/results.

Auto-Placement of Reagent Indexes

The Clarity LIMS NGS Extensions Package includes the `place_indexes` script, which assigns indexes (reagent labels) to samples in the destination container, based on the pattern defined in a specified index pattern file—and optionally validates the indexes against the pattern file.

This section provides details on the script and its parameters, and LIMS configuration requirement

Script Overview

The `place_indexes` script is intended to be run on entry to, and optionally on exit from, the Add Labels / Reagents screen of a step.

On entry to Add Labels / Reagents screen, the script assignes an index (label) to each sample in the destination container, based on the pattern defined in the specified index pattern file.

On exit from Add Labels / Reagents screen, the script validates the indexes against the pattern file. You can also use the script to check:

- If the user has modified the indexes.

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- If the current step permits user modifications.

Script Parameters

-u {username} -username {username}	(Required) LIMS login username
-p {password} -password {password}	(Required) LIMS login password
-i {stepURI:v2} -stepURI {stepURI:v2}	(Required) LIMS step URI
-f <pattern file directory and file name> -patternFilePath <pattern file directory and file name>	(Required) Path (directory + file name) of index placement pattern files Providing the directory without the file name results in an error. You must provide both.
-ic <'name of reagent category / label group'> -ignoredCategory <'name of reagent category / label group'>	(Optional, may be provided multiple times) Reagent category for which autoplacement should not occur
-locked <true/false>	(Optional) Provide as true if the index placement is locked and not modifiable by the user. If not provided, defaults to false.
-fc <true/false> -finalCheck <true/false>	(Optional) Provide as true if you are running this as the final check to make sure autoplacement was not modified.

Command Line Example

```
bash -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/PlacementHelper.jar \
-i {stepURI:v2:http} \
-u {username} \
-p {password} \
script:place_indexes \
-f /opt/gls/clarity/extensions/conf/infinium/indexplacementpatterns \
-ic 'NoIndex' \
-locked true \
-fc true"
```

Note that this command line also uses the Sample Placement Helper script (PlacementHelper.jar), included in the Lab Instrument Toolkit.

Pattern File

The index pattern file is a tab-separated file consisting of two columns, DEST_WELL and INDEX_NAME, and is in the following format:

DEST_WELL	INDEX_NAME
A:1	AD001 (ATCACG)
B:1	AD002 (CGATGT)
C:1	AD003 (TTAGGC)
D:1	AD004 (TGACCA)
E:1	AD005 (ACAGTG)
F:1	AD006 (GCCAAT)
G:1	AD007 (CAGATC)
H:1	AD008 (ACTTGA)
A:2	AD009 (GATCAG)

The destination well format provided in the file must match the well placement configuration of the container type intended for use in the LIMS. The reagent index name must match the name of a reagent in the LIMS.

Pattern name files may be customized. However, for out-of-the-box LIMS integrations, the supplied pattern file names follow the convention: placement_pattern_index_xx_yy.tsv, where:

- xx is a short description about the step that accesses this file
- yy is a 2-digit value in the range from 01 to 99

Validation

There are three levels of validation provided by the script:

- Index placement can be modified by the user on the fly.
- Index placement is locked down by an administrator and cannot be modified by the user.
- Index placement must be locked down by an administrator prior to use.

Two options determine which level of validation to apply:

- The presence of a corresponding checksum file for the given pattern file.
- The presence of the locked script parameter.

When there is no checksum file, and locked is not provided to the script (or is provided as false), index placement is user-modifiable.

Checksum file

If the reagent index placement pattern is not user-modifiable, an administrator can create a checksum file to ensure that the user has not changed the index placement on the fly.

This checksum file should have the same name as the placement pattern file, but with the file extension .md5. For example:

- placement_pattern_index_pcramp_01.tsv
- placement_pattern_index_pcramp_01.md5

The checksum file must be placed in the same location as the corresponding index pattern file. When a checksum file is found and the user has modified the indexes on the samples in the step, the script reverses the changes made by the user.

The checksum file contains the .md5 checksum for the corresponding pattern file itself. This checksum value is compared against the pattern file to validate whether the pattern file has been modified. If it has been modified, the script displays an error message and index placement does not occur. If an administrator wishes to change the pattern file contents, they must also regenerate the checksum when the updated pattern is available for use.

Locked patterns

The `locked` parameter provides an additional layer of validation. If this is provided to the script, the current pattern requires the presence of a checksum file. This ensures that an administrator has approved the pattern for use before it is used in a step.

Ignored categories

In some steps where reagent autoplacement is configured, there may be some reagent categories where this is not desired.

For example, the workflow may permit either a full 96 well plate of samples in the indexing step, or merely 8 samples in tubes. For the 8 samples, a different reagent category is used than for the batch of 96. In this case, the `ignoredCategory` parameter may be used to indicate that if the 8-sample reagent category is selected for the step, index placement will be performed manually.

Configuration

The place_indexes script is intended to be run on entry to / exit from the Add Labels / Reagents screen of a step.

To achieve this, configure the automation trigger as follows:

LIMS v5.x

- Trigger Location: Add Labels screen
- Trigger Style: Automatic upon entry / exit

LIMS v4.x

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- Trigger Behavior: Automatically initiated
- Stage of Step: on Add Reagents screen
- Timing: When screen is entered / exited

Rules and Constraints

- The step must be configured for adding reagent indexes.
- The script must run on entry to or exit from the Add Labels / Reagents screen.
- The pattern file must be a tab-separated (.tsv) file.
- The pattern file headers must be DEST_WELL and INDEX_NAME.
- The reagents in the pattern file must match the name of reagents configured in the LIMS.
- Only Index type reagents are supported.
- The destination container type selected must match the well placement information in the pattern file.
- There must not be more samples in the step than are supported by the selected pattern file.
- Only a single destination container is supported.
- If the locked parameter is provided, a checksum file must be present.
- If a checksum file is present, the index placements may not be modified by the user.
- The checksum file must be in the same location and have the same name as the pattern file, with extension .md5.
- The checksum file contents must be the checksum value for the corresponding pattern file.
- If a checksum file is present, the pattern file and checksum file must have the same checksum value.

Compute Replicate Average

The Clarity LIMS NGS Extensions Package v5.14 includes the computeReplicateAverage script, which automates calculation of replicate (referred to as derivatives in the LIMS) average values. The script can also optionally calculate standard deviation and %CV values.

This section provides details on the script, its parameters, and LIMS configuration requirements.

Script Overview

The `computeReplicateAverage` script uses data stored in a source UDF / custom field (specified by the src parameter) to calculate the average across replicates, and writes the resulting value to a destination UDF / custom field (specified by the dest parameter).

- The script assumes the step has input analytes / derived samples with replicated per-input results / measurements.

- The script groups the per-input results / measurements by their parent analyte / derived sample.
- For each group, the script calculates the average of the result UDFs / measurement custom fields specified by the src parameter, ignoring any results that:
  - Are missing `src` values, or
  - Have an `exclude` field set to true
- The resulting averages are written back into the parent analyte / derived sample field specified by the `dest` parameter for each group.
- The `exclude` parameter is optional and no exclusions are performed if it is missing.

The script can also be used to calculate standard deviation and %CV values and add these results back to the corresponding UDF / field in the parent analyte / derived sample. The names of the Standard Deviation and %CV UDFs must be given to the script for it to update the values.

Script Parameters

-u {username} -username {username}	(Required) LIMS login username
-p {password} -password {password}	(Required) LIMS login password
-i {stepURI:v2} -stepURI {stepURI:v2}	(Required) LIMS step URI
-log {logFileLIMSID} -logFileName {logFileLIMSID}	(Required) LIMS ID of the log file placeholder
-src <srcUDF> -srcUDF <srcUDF>	(Required) Source UDF / custom field name
-dest <destUDF> -destUDF <destUDF>	(Required) Destination UDF / custom field name
-exclude <excludeUDF> -excludeUDF <excludeUDF>	(Optional) Exclude UDF / custom field name
-sd <sdUDF> -stdDevUDF <sdUDF>	(Optional) Standard deviation UDF / custom field name
-cv <cvUDF> -cvUDF <cvUDF>	(Optional) %CV UDF / custom field name

Command Line Example

When working with the `computeReplicateAverage` script, the automation command line must be constructed as follows:

```
bash -l -c "/opt/gls/clarity/bin/java -jar \
```

```
/opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar \
-i {stepURI:v2} \
-u {username} \
-p {password} \
script:computeReplicateAverage \
-src '<source field name>' \
-dest '<destination field name>' \
-exclude '<Exclude field name>'
-log {<log file name>}"
```

Example 1:

```
script:computeReplicateAverage \
-src 'Concentration' \
-dest 'Avg Concentration' \
-exclude 'Ignore' \
-log {compoundOutputFileLuid0}"
```

In this example, the script will:

1. Average the values captured in the replicate Concentration fields. Replicates whose Ignore field value is set to True will be excluded from the calculation.
2. Copy the result to the Avg Concentration field configured on the parent derived sample.

Example 2:

```
script:computeReplicateAverage \
-src 'Concentration' \
-dest 'Avg Concentration' \
-sd 'Std Dev' \
-cv '%CV' \
-exclude 'Ignore' \
-log {compoundOutputFileLui
```

In this example, the script will:

1. Average the values captured in the replicate Concentration fields. Replicates whose Ignore field value is set to True will be excluded from the calculation.
2. Copy the replicate average result to the Avg Concentration field configured on the parent derived sample.
3. Calculate standard deviation and %CV values.
4. Copy the standard deviation and %CV results to the Std Dev and %CV fields configured on the parent derived sample.

Example 3

```
script:computeReplicateAverage \
-src 'Raw CP' \
```

```
-dest 'Raw CP' \
-log {compoundOutputFileLuid0}"
```

In this example, the script will:

1. Average the values captured in the replicate Raw CP fields. No replicates will be excluded from the calculation.
2. Copy the result to the Raw CP field configured on the parent derived sample. No values will be ignored

Configuration

The inputs to the step must have values for the analyte UDF / derived sample custom field to be used as the source field. There must be an additional analyte UDF / derived sample custom field configured as the destination field - to capture the calculated replicate average. Both field types must be Numeric.

The following table provides example field configuration details.The field names used correspond to those used in the command line Example 1 shown above.

Field Name	Field Type	Field Constraints / Options	Preset Values / Additional Options
Configured on ResultFile in LIMS v4.2.x and Measurement in LIMS v5.x & later:			
Concentration In example 1, this is the field specified by the -src parameter	Numeric	• Required: Yes • Read Only: No	Decimal Places Displayed: 2
Ignore In example 1, this is the field specified by the -exclude parameter	Boolean (v4.2.x) Toggle Switch (v5.x)	• Required: No • Read Only: No	Default: None Set
Configured on Analyte in LIMS v4.2.x and Derived Sample in LIMS v5.x & later:			
Avg Concentration In example 1, this is the field specified by the -dest parameter	Numeric	Required: Yes Read Only: No	Decimal Places Displayed: 2

Copy UDFs

Compatibility: Clarity LIMS v2.0.5 or later; NGS Extensions Package v4.0 or later

Often, a user has certain values associated with libraries prior to preparing a flow cell. When loading the libraries into the flow cell, the user needs to track these values as attributes of the flow cell lane to help interpret the metrics generated during sequencing.

For example, one might need to record a concentration as a sample UDF on normalized and pooled libraries. This can be accomplished by copying the value of identically named UDFs from inputs to non-shared outputs.

Script Overview

The `copyUDFs` script provides the ability to selectively copy the value of an input UDF to a UDF of the same name on outputs generated by a protocol step (process). Other capabilities of this script include:

The ability to map a single input to one or more outputs

The ability to define which UDF is copied across to outputs

Script Parameters

-u {user	(Required) LIMS login username
-p {password}	(Required) LIMS login password
-i {stepURI:v2}	(Required) LIMS step URI
-f {name, name, name}	(Required) UDF names (Required)

Constraints

- Only the UDFs named in the -f (fields) parameter will be copied.
- If input UDFs are not defined or have no value, nothing will be copied for those UDFs.
- UDFs not being defined on an output result in the script aborting after displaying an error message.
- The script is not applicable to shared outputs.
- UDFs of the same name must be present in the input and enabled as an output detail.

Initialize Artifact UDFs

Compatibility: Clarity LIMS v2.0.5 or later; NGS Extensions Package v4.0 or later

This section discusses using the `initArtifactUDFs` script to transfer the UDFs associated with samples and processes to downstream artifacts. Typically, the script is used to copy UDFs from sample inputs and processes to sample outputs.

Script Overview

For each output, the `initArtifactUDFs` script searches for UDFs of the same name - in both the input samples and the process. If a match is found, it then copies the UDF values to the corresponding UDFs on the sample output.

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How it works:

1. Identify the list of sample UDFs configured as output UDFs for this process (e.g. as would be defined in the 'Output Details' section of the process configuration).
2. For each sample UDF, look for an identically named UDF on the submitted sample; if a match exists, copy the value from the submitted sample to the sample's output UDF. If there is no UDF match, do nothing.
3. For each sample UDF, look for an identically named UDF on the process; if a match exists, copy the value from the process to the sample's output UDF. If there is no UDF match, do nothing.
4. Typically, the values will be derived from the submitted sample(s). In some cases, there will also be a need to copy data from the process level (specified by the user executing the process) down to each sample output UDF.
5. In some cases, there may be a need to account for pooling - i.e., where there are multiple submitted samples for a single output sample. The basic strategy here is to create small groupings of these values, such that each grouping does not contain multiple entries of the same UDF value.

Useful information

- If both process and any number of samples have values for the same UDF name, the process UDF value is used.
- If multiple samples have different values for the same UDF name, the values are combined using '+' as a separator (up to 5 values, additional values are omitted and represented with '...').
- In the case where an output UDF value already exists, the initArtifactUDFs script will NOT override existing values. (This scenario is extremely rare - one has to be very fast to manually set UDF values before an EPP script runs.)

Script Parameters

-u {user	(Required) LIMS login username
-p {password}	(Required) LIMS login password
-i {URI}	(Required) LIMS process URI

Command Line Example

```
bash -l -c "/opt/gls/clarity/bin/java -jar \
/opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar \
script:initArtifactUDFs \
-u {username} \
-p {password} \
-i {processURI:v2}"
```


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In the example shown above, the paths may differ for your system. For example, the version number may differ.

Label Non-Labeled Outputs

Compatibility: Clarity LIMS v2.0.5 or later

A user may need to add reagent labels to sample outputs that do not have reagent labels assigned to them.

Script Overview

The `labelNonLabeledOutputs` script provides the ability to add reagent labels to sample outputs for which there is no pre-existing reagent label.

The script checks each process output for existing reagent labels; if an output does not have at least one pre-existing reagent label then the label supplied as a script parameter is applied.

- For each output that does not already have a reagent label applied to it (e.g. control samples), the script will apply a reagent label named with the supplied string.
- In the Preconfigured Package for Illumina NGS, all sequencing libraries go through a demultiplex process to both convert data to FASTQ and demultiplex reads if necessary. Note that presently, inputs that do not have a reagent label do not create an output in a demultiplex process even though we need BCL convertor to convert their reads to FASTQ.

Script Parameters

-u {user	(Required) LIMS login username
-p {password}	(Required) LIMS login password
-i {URI}	(Required) LIMS process URI
-l {name}	(Required) Reagent label to be applied

Command Line Example

```
java -jar ngs-extensions.jar script:labelNonLabeledOutputs -u admin -p pass -i
http://localhost:8080/api/v1/processes/RCX-RCX-110315-24-3 -l "GENERIC_INDEX"
```

You may also find the following script useful: *Initialize Artifact UDFs* on page 2571

Linear Regression Calculation

Clarity LIMS NGS Extensions Package v5.17 and later includes `computeLinearRegression` script. This script calculates values for slope, intercept, and r-squared, and uses these values to populate corresponding fields. The field names are configurable via script parameters, and will differ depending on the LIMS integration.

In addition, the script requires values for y-axis and x-axis. These values are both related to control samples:

- y-axis: This is configured as an analyte UDF / derived sample custom field on the control sample. The field name is configurable and is passed in as a script parameter.
- x-axis: This is the control type's concentration (entered in the Conc. field). This is not configurable.

Script Parameters

The following table defines the parameters used by the `computeLinearRegression` script.

-u {username} -username {username}	(Required) LIMS login username
-p {password} -password {password}	(Required) LIMS login password
-i {stepURI:v2} -stepURI {stepURI:v2}	(Required) LIMS step URI
-log {logFileLIMSID} -logFileName {logFileLIMSID}	(Required) LIMS ID of the log file placeholder
-y <FieldName> -yAxisUDF <UDFName>	(Required) Analyte / Derived sample field name for Y axis.
-slope <FieldName> -slopeUDF <FieldName>	(Required) Slope process / step field name
-ic <FieldName> -interceptUDF <FieldName>	(Required) Intercept process / step field name
-rsq <FieldName> -rSquaredUDF <FieldName>	(Required) R-squared process / step field name

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-x <FieldName> -xAxisField <FieldName>	(Deprecated parameter, not supported) Control type concentration field name for x axis. This value specifies the API name that will be used to read the concentration from the control type. Not configurable. Note: This field appears as concentration in the API, but displays as Conc. in the user interface. You cannot customize this naming in the API. In addition, the script does not attempt to read the concentration from the control sample instead of the control type. For example, if you add a UDF / custom field to the control sample, you cannot use that field as the X axis value.
-logX <true/false> -logXMode <true/false>	(Optional) If set to 'true', reads the concentration value from the control type, calculates the log10 of that value, and uses the result for the x-axis. • Default = false
-minData <#> -minDataPts <#>	(Optional) Minimum number of data points needed to run linear regression. • Default = 4
-select <(ControlName1)|(ControlName2)> -selectControl <(ControlName1)|(ControlName2)	(Optional) Regular expression that selects the control names to be used in the calculation. • Default = All controls

Command Line Example

```
bash -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar \
-i {stepURI:v2} \
-u {username} \
-p {password} \
-log {compoundOutputFileLuid0} \
script:computeLinearRegression \
-y 'Raw CP' \
-slope 'Slope' \
-ic 'Intercept' \
```


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```
-rsq 'R-Squared' \
-logX 'true' \
-minData '4' \
-select '(Control Name 1)|(Control Name 2)'"
```

Configuration

This section describes the configuration required for the linear regression calculation.

Step UDFs /Custom Fields

Three step UDFs / custom fields must be configured on the master step / process type, to capture the linear regression slope, intercept, and r-squared values. These fields should display on the Record Details screen at run time.

The following table provides example field configuration details. The field names used correspond to those used in the command line example shown above.

Field Name	Field Type	Field Constraints / Options
Intercept The field specified for the -ic parameter	Numeric	• Required: No • Read Only: No • Custom Entries: Yes
R-Squared The field specified for the -rsq parameter	Numeric	• Required: No • Read Only: No • Custom Entries: Yes
Slope The field specified for the -slope parameter	Numeric	• Required: No • Read Only: No • Custom Entries: No

Global Custom Fields / UDFs

The control samples in the step must have values for a specific derived sample custom field / analyte UDF.

The following table provides example configuration details for this field. The field name used corresponds to the name used in the command line example above.

The field is configured on Analyte in LIMS v4.2.x and Derived Sample in LIMS v5.x.

Field Name	Field Type	Field Constraints / Options
Raw CP The field specified for the -y parameter	Numeric	• Required: No • Read Only: No • Custom Entries: Yes

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Typically it is the step outputs that need to have this field available to fill in. However, the field could also be made available on the step inputs to support the case where multi-step controls are used as inputs to the step.

Rules and Constraints

- Control samples used in the step must have valid concentration values:
  - Concentration must be a numeric value.
  - Optionally, the numeric concentration value can have text following it to specify the units, for example - 10.02 nM. Note that units are for display purposes only and are not factored into the calculation.
  - Concentration must be a value other than 0. Controls with 0 concentration value are ignored.
- Slope, intercept, r-squared and -y (Raw CP in the example) field values must all be numeric.
- There must be 4 or more control samples in the step that can be used in the calculation - i.e., after passing validation.
  - Number of control samples can be configured through -minData parameter.
- Control samples must have:
  - A name that matches the pattern provided by the -select parameter (if provided).
  - A y-axis field filled in with a numeric value.
  - A concentration value filled in for their control type, with a non-zero numeric value.
  - If any of these conditions is not met, the control samples fail validation and are excluded from the calculation. These do not count towards the minimum number of control samples.
- If -logX parameter is specified as 'true', the calculated log10(concentration) value is used as the x-axis value (see Script parameters).

Normalization Buffer Volumes

Compatibility: Clarity LIMS v1.0 or later; NGS Extensions Package v4.1 or later

This section discusses the `normalizationBufferVolumes` script, which is used to calculate buffer volumes.

Script Overview

The `normalizationBufferVolumes` script generates a comma-separated file—Normalization buffer volumes.csv— file that holds the calculated buffer volumes information. The generated file is a attached to the Library Normalization step in the LIMS.

The script calculates the 'Volume of Dilution Buffer (µL)' as follows:

- "Volume of Dilution Buffer (µL)" = (("Source Volume (µL)" * "Source Conc.") / "Destination Conc.") - "Source Volume (µL)"

For Research Use Only. Not for use in diagnostic procedures.

How it works:

- The script requires that input samples to the step have Concentration and Conc. Units UDFs.
- The only supported value for Conc. Units is nM.

Script Parameters

-u {user	(Required) LIMS login username
-p {password}	(Required) LIMS login password
-i {URI}	(Required) LIMS process URI
-c {filename}	Normalization Buffer Volumes CSV file name (Required)

Command Line Example

```
bash -l -c "/opt/gls/clarity/bin/java -jar \
/opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar
script:normalizationBufferVolumes \
-i {processURI:v2:http} \
-u {username} \
-p {password} \
-c {compoundOutputFileLuid0}
```

Configuration

The following step UDFs are configured on the Library Normalization step:

- Library volume (µL) transferred to DESTINATION plate: Numeric, Default = 10
- Normalized conc. (nM): Numeric, Default = 2
- Maximum destination container volume (µL): Numeric, Default = 800

The inputs to the step must have values for the following sample UDFs:

- Concentration: Numeric
- Conc. Units: Single Line Text

Rules & constraints

- One sample input generates one sample output and one shared result file.
- The CSV file header contains the date and a record of the user who generated the file.
- The contents of the CSV file are ordered by source plate and then source well.

Additional information

Other scripts you may find useful:

Initialize Artifact UDFs on page 2571

Process Summary Report

Compatibility: Clarity LIMS v2.0.5 - v4.2

Often, a user wants to determine what work has been performed on several samples by running the Sample History Report. However, suppose they were to use the sampleHistoryReport script on 600 samples that involve the same four processes. The result would be 600 reports (one for each sample), each of which provides information on only that one sample.

A preferable alternative is to use the processHistoryReport script, which is run only once for each process. In this example, therefore, it would produce only four reports (one for each process) allowing for a better grouping of information and less files.

Script Overview

The `processHistoryReport` script has two parts: 1. Retrieve the necessary information, and 2. Compile that information into a report.

- The script generates one report ('Process Summary Report') for all inputs, output as a single shared result file.
- The script is configured on the Illumina Sequencing (Illumina SBS) 5.0 step, as 'Report creation script.'
- The report is generated in XLS format using POI.
- The report is automatically attached to the pre-configured report process result file output.

Script Parameters

-u {user	(Required) LIMS login username
-p {password}	(Required) LIMS login password
-i {URI}	(Required) LIMS process URI

Adding Processes to a Report

1. Add a process UDF of type Checkbox, with the exact name of the process you want to include. For example: "3. Denature, Dilute and Load Sample (MiSeq)"
2. When the process is run, select the checkbox to include that process in the Process Summary Report.

```
OUTPUT GENERATION NAMING EXAMPLE:
```

```
Process History Report

EPP PARAMETER STRING EXAMPLE:
java -jar ngs-extensions.jar script:processHistoryReport -u {username} -p
{password} -i {processURI}
```

Note that the script may also be run in the following directory:

```
/opt/gls/clarity/extensions/ngs-common/v4/EPP
```

Configuration

Report process configuration may be obtained using the LIMS configuration import/export tools or the LIMS Config-Slicer tool.

Rules & constraints

- The script must be run from the LIMS Operations Interface.
- To be included in the report, a process must have at least one output UDF specified.
- The report requires a pre-configured process type + EPP parameter string ('TruSeq Process Summary Report').
- The report runs from the specified artifact down the genealogy tree. For complete and correct output, it is recommended that it be run on the original submitted samples.

Additional information

Other scripts you may find useful:

- *Set UDF* on page 2586
- *Copy UDFs* on page 2570

Routing Script

The routing script allows for the movement of samples from one workflow to another. The script can move sample individually or in bulk.

- Individually—the script uses derived sample custom fields (analyte UDFs in v4.x)
- In bulk—the script uses master step custom fields (step UDFs in v4.x).

To move samples, first define the workflow and the step to which the samples will be sent. The script can be modified in the automation command line.

This section discusses the Routing script - Normalize Libraries automation used in the Preconfigured Workflow Package (PCWP). The automation invokes the `changeWorkflow` script, which routes step outputs to the next protocol in the workflow according to the Sequencing Instrument field value of each sample.

For Research Use Only. Not for use in diagnostic procedures.

Automation Configuration

Command Line

The default automation command line for the routing script is as follows.

```
bash -c "/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -u {username} -
p {password}
-i {stepURI:v2} -l {compoundOutputFileLuid0} script:changeWorkflow \
\
--FIELD_NAME 'Name of the field' \
--FIELD_VALUE 'Preset value of the field' \
--WORKFLOW 'Name of the workflow' \
--STEP 'Name of the Step in the workflow \
--INPUTS_OR_OUTPUTS 'One or the other'"
```

The default command line includes one section, and will always send the samples to the same workflow. To offer multiple workflow and/or step choices, you must add multiple sections, as shown in the following examples.

To send samples into a QC Protocol, use the protocol name instead of the name of the step name.

Script Parameters

-i {lims step uri} -stepURI {lims step uri}	LIMS step URI (Required)
-u {username} -username {username}	LIMS login username (Required - requires admin credentials)
-p {password} -password {password}	LIMS login password (Required)
-l <log file path and name> -logFileName <log file path and name>	Path destination for the log file (Required)
--FIELD_NAME	LIMS custom field name (derived sample (analyte) or step)
--FIELD_VALUE	LIMS UDF preset /custom field option value If the field type is a check box/toggle, the --FIELD_VALUE should be set as 'true' or 'false''

--WORKFLOW	Name of LIMS workflow to which the samples will be sent
--STEP	LIMS step within the workflow
--INPUTS_OR_OUTPUTS	Specifies whether the LIMS UDF / custom field is from the input or the output

Example 1

Routing script configured on Normalize Libraries steps in PCWP v1.0

```
bash -c "/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -u {username} -
p {password}
-i {stepURI:v2} -l {compoundOutputFileLuid0} script:changeWorkflow \
\
--FIELD_NAME 'Sequencing Instrument' \
--FIELD_VALUE 'MiSeq' \
--WORKFLOW 'MiSeq Sequencing v1.0' \
--STEP 'Library Pooling (MiSeq v1.0)' \
--INPUTS_OR_OUTPUTS 'OUTPUTS' \
\
--FIELD_NAME 'Sequencing Instrument' \
--FIELD_VALUE 'NextSeq' \
--WORKFLOW 'NextSeq 500/550 Sequencing v1.0' \
--STEP 'Library Pooling (NextSeq 500/550 v1.0)' \
--INPUTS_OR_OUTPUTS 'OUTPUTS' \
\
--FIELD_NAME 'Sequencing Instrument' \
--FIELD_VALUE 'HiSeq2500' \
--WORKFLOW 'HiSeq 2500 Sequencing v1.0' \
--STEP 'Library Pooling (HiSeq 2500 v1.0)' \
--INPUTS_OR_OUTPUTS 'OUTPUTS' \
\
--FIELD_NAME 'Sequencing Instrument' \
--FIELD_VALUE 'HiSeq3000/4000' \
--WORKFLOW 'HiSeq 3000/4000 Sequencing v1.0' \
--STEP 'Library Pooling (HiSeq 3000/4000 v1.0)' \
--INPUTS_OR_OUTPUTS 'OUTPUTS' \
\
--FIELD_NAME 'Sequencing Instrument' \
```


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```
--FIELD_VALUE 'HiSeqX' \
--WORKFLOW 'HiSeq X Sequencing v1.0' \
--STEP 'Library Pooling (HiSeq X v1.0)' \
--INPUTS_OR_OUTPUTS 'OUTPUTS' \
\
--FIELD_NAME 'Sequencing Instrument' \
--FIELD_VALUE 'NovaSeq' \
--WORKFLOW 'NovaSeq 6000 v2.0' \
--STEP 'Define Run Format (NovaSeq 6000 v2.0)' \
--INPUTS_OR_OUTPUTS 'OUTPUTS'"
```

Example 2

Routing script configured on DNA/RNA Extraction step in TruSight Tumor 170 v1.0 workflow

```
bash -l -c "/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -u {username} -
p {password}
-i {stepURI:v2} -l {compoundOutputLuid0} script:changeWorkflow \
\
--FIELD_NAME 'Sample Type' \
--FIELD_VALUE 'RNA' \
--WORKFLOW 'TruSight Tumor 170 v1.0' \
--STEP 'Qubit RNA QC (TruSight Tumor 170 v1.0)' \
--INPUTS_OR_OUTPUTS 'OUTPUTS' \
\
--FIELD_NAME 'Sample Type' \
--FIELD_VALUE 'DNA' \
--WORKFLOW 'TruSight Tumor 170 v1.0' \
--STEP 'Qubit DNA QC (TruSight Tumor 170 v1.0)' \
--INPUTS_OR_OUTPUTS 'OUTPUTS'"
```

Configuration Requirements

Next Step Configuration

For the routing script to be triggered, the Next Step value for all samples in the step must be set to Remove from workflow. You can set this value manually or automatically, using a script.

Set the next step manually:

At run time, on the Assign Next Steps screen, the lab scientist sets the Next Step value to Remove from workflow.

The Remove from workflow option is only available on the Assign Next Steps screen for users who have the RemoveSampleFromWorkflow permission. If you use the manual method, you must make sure that users who need to set this option at run time have the correct permission. For details on configuring role-based permissions, see the core LIMS documentation.

Set the next step automatically:

Add the following script to an automation command line enabled on the step. Configure the protocol and set the Assign Next Step function to Automatic.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar
-i {stepURI:v2} -u {username} -p {password} script: evaluateDynamicExpression -t false -h false
-exp 'nextStep = ::REMOVE::' -log {compoundOutputFileLuid0}"
```

LIMS v5.x

On the protocol configuration screen, in the Next Steps section, the method of assigning the next step must be set to Automatic.

LIMS v4.2.x

In the protocol configuration area, on the Next Steps configuration tab, the Next Steps are set by a script toggle switch. Set this switch to Yes.

Automation Trigger Configuration

By default, the routing script automation should be configured to be automatically triggered at the end of the step.

After the script has completed, a dialog box displays. This dialog box shows the number of samples routed to each step—or an error message if something went wrong.

Global Custom Fields / UDFs

The following table provides configuration details for the global custom field configured to support the routing script automation. This example is based on the Routing script - Normalize Libraries automation.

The field is configured on Analyte in LIMS v4.2.x and Derived Sample in LIMS v5.x.

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Field Name	Field Type	Field Constraints / Options	Preset Values / Dropdown Items
Sequencing Instrument	• Single-line Text (v4.2.x) • Text Dropdown (v5.x)	• Required: Yes • Read Only: No • Custom Entries: No	• MiSeq • NextSeq • HiSeq2500 • HiSeq3000/4000 • NovaSeq • HiSeqX

Modifying the Default Configuration

You can modify the default configuration by removing / adding instruments.

To remove an instrument:

1. Remove the unwanted FIELD_VALUE from the list of presets.
2. In the automation command line, remove the section that corresponds to the unwanted FIELD_VALUE.

To add an instrument:

1. Add a field option / preset for the new FIELD_VALUE.
2. Add a section in the routing script command line to set the parameters for the additional FIELD_VALUE.

For example, to add "New instrument" to the Routing script - Normalize Libraries automation, add the following to the end of the script:

```
\
--FIELD_NAME 'Sequencing Instrument' \
--FIELD_VALUE 'New instrument' \
--WORKFLOW 'Workflow name for New instrument' \
--STEP 'Step of the Workflow for New instrument' \
--INPUTS_OR_OUTPUTS 'OUTPUTS'"
```

Rules and Constraints

- The --FIELD_NAME and --FIELD_VALUE are only available for derived sample and master step custom fields.
- If no custom field should be checked, provide "N/A" to FIELD_NAME and FIELD_VALUE.
- If the --FIELD_VALUE parameter value passed to the script does not match the value in the --FIELD_NAME custom field, the routing script does not route the sample.

- The routing script works with the Remove from workflow function in the Next Steps of any step. Make sure that the correct settings or permissions are in place to allow for this function. For details, see the preceding Configuration Requirements section.

Set UDF

Compatibility: Clarity LIMS v2.0.5 or later; NGS Extensions Package v4.0 or later

Often, a user would like to set or change the value of a UDF. If the UDF is accessible from the initial process URI, the setUDF script can be used.

Script Overview

The `setUDF` script allows several operations to take place, including - but not limited to the following:

- Set a UDF on an output container to today's date.
- Set a UDF on any sample associated with any input to a person's name.
- Set a UDF on any output to a lab name (referred to as 'Account' in Clarity LIMS).
- Set the input container name as a process level UDF.
- Read a project UDF and set it on a sample.

Script Parameters

-u {user	(Required) LIMS login username
-p {password}	(Required) LIMS login password
-i {URI}	(Required) LIMS process URI
-f {name}	UDF names (Required)
-t {XPath}	Path to the target object (Required)
-s {XPath}	Path to the source element/attribute (Optional)
-v {value}	UDF value (Required if the path to the source value is not provided, optional otherwise)

Examples

There are multiple ways to implement this script, as illustrated by the following examples.

Example 1

Set the UDF named MyUDF on any output containers to the date the process ran:

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```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar \
script:setUDF \
-u {username} \
-p {password} \
-i {processURI:v2} \
-f 'MyUDF' \
-t '//output/@uri->//container/@uri' \
-s '//date-run'"
```

Example 2

Set the UDF named MyUDF on any sample associated with any input to the name of the technician:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar \
script:setUDF \
-u {username} \
-p {password} \
-i {processURI:v2} \
-f 'MyUDF' \
-t '//input/@uri->//sample/@uri' \
-s '//technician/*'"
```

Example 3

Set the UDF named MyUDF on any output to the name of the technician's lab:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar \
script:setUDF \
-u {username} \
-p {password} \
-i {processURI:v2} \
-f 'MyUDF' \
-t '//input/@uri' \
-s '//technician/@uri->//lab/@uri->//name'"
```

Example 4

Set the input container name as a process level UDF:

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar \
```


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```
script:setUDF \
-u {username} \
-p {password} \
-i {processURI:v2} \
-f 'FlowCellID' \
-t '/process/@uri' \
-s '//input/@uri->//container/@uri->//name'"
```

Example 5

Read a project UDF and set it on a sample:

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar \
script:setUDF \
-u {username} \
-p {password} \
-i {processURI:v2} \
-f 'Study' \
-t '//input/@uri->//sample/@uri' \
-s '//input/@uri->//sample/@uri->//project/@uri->/project/field[@name="Study"]'
```

Example 6

Read a parent process UDF and set it on a sample level UDF called Geneticist:

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar \
script:setUDF \
-u {username} \
-p {password} \
-i {processURI:v2} \
-f 'Geneticist' \
-t '//input/@uri->//sample/@uri' \
-s '//input/@uri->//parent-process/@uri->//technician/@uri->//initials'
```

Example 7

Read the Project name and set it on a sample level UDF called ProjectName:

```
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar \
script:setUDF \
-u {username} \
```

```
-p {password} \
-i {processURI:v2} \
-f 'ProjectName' \
-t '//input/@uri->//sample/@uri' \
-s '//input/@uri->//sample/@uri->//project/@uri->//name'
```

In the examples shown above, the paths may differ for your system.
For example, the version number may differ. For Clarity LIMS 4 or before, the process URI token should be: `{processURI:v2:https} or {processURI:v2:http}`

Rules & constraints

- Both the target entity and (optionally) the source value are expressed as chains of XPath expressions (using -> as separators).
- Even if you are using XPath to specify the source value, setUDF applies a single value per invocation to all target objects. It cannot apply different source values to different target objects.
- You can use -z as an option when running the script to simulate the effects it would have. Note that even though an output identifying the target object(s) and the value that would be set is produced no changes are actually made.

Additional information

Other scripts you may find useful:

- *Initialize Artifact UDFs* on page 2571
- *Copy UDFs* on page 2570

Validate Complete Plate

Clarity LIMS NGS Extensions Package v5.17 and later includes the `validateCompletePlateStarted` script. This script checks that all samples in a container were started together on the current step. This section provides details on the script and its parameters, and LIMS configuration requirements.

Script Overview

The purpose of the `validateCompletePlateStarted` script is to make sure that all of the samples in the input plate were started together. The validation supports partially-filled plates and multiple plates.

You can supply additional parameters to the script, to optionally enforce:

- 'Cannot proceed with partial plate' logic for only for the samples that are queued to the current step, instead of the entire plate contents. Default is false—the entire plate contents are verified.
- Contiguous row-major or column-major layout. This makes sure that the input plate(s) are in the expected layout and contain no gaps between the first and last sample. By default, this is not enforced.

For Research Use Only. Not for use in diagnostic procedures.

Script Parameters

-u {username} -username {username}	(Required) LIMS login username
-p {password} -password {password}	(Required) LIMS login password
-i {stepURI:v2} -stepURI {stepURI:v2}	(Required) LIMS step URI
-log {logFileLIMSID} -logFileName {logFileLIMSID}	(Required) LIMS ID of the log file placeholder
-layout <'ROW'/'COLUMN'>	(Optional) One of 'ROW' or 'COLUMN', if provided. When present, script enforces that the samples are laid out in gap-free, row-major or column-major order.
-queuedOnly <true/false>	(Optional) When provided and set to true, the script ensures that samples queued to the started step were started together. Samples dropped earlier in the workflow will are ignored. Default is false.
-t <true/false> -testMode <true/false>	(Optional) When provided and set to true the script reports errors in accordance with 'Record Details button' mode, instead of the default 'screen transition' mode. Default is false.

Command Line Examples

Example 1

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar \
-i {stepURI:v2} \
-u {username} \
-p {password} \
-log {compoundOutputFileLuid0} \
script:validateCompletePlateStarted"
```

Example 2

```
bash -l -c "/opt/gls/clarity/bin/java -jar/opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar \
-i {stepURI:v2} \
```

```
-u {username} \
-p {password} \
-log {compoundOutputFileLuid0} \
script:validateCompletePlateStarted" \
-layout 'ROW' \
-queuedOnly true -t true
```

Configuration

The `validateCompletePlateStarted` script is intended to run when the user selects the Begin Work button on the Ice Bucket screen and starts the step.

To achieve this, configure the automation trigger as follows:

- Trigger Location: Step
- Trigger Style: Automatic upon entry

Validate Sample Count

The Clarity LIMS NGS Extensions Package includes the `validateSampleCount` script, which checks the number of samples being input to or output by the step.

This article provides details on the script and its parameters, and LIMS configuration requirements.

Script Overview

The script validates the number of step inputs or outputs, comparing it against configurable minimum and maximum values - determined by -min and -max parameters.

By default, the script runs in count inputs mode and validates the step inputs. If you supply the optional -o parameter with value set to true, the script runs in count outputs mode and validates the step outputs.

Count inputs mode

The typical use case for count inputs mode is to ensure that the correct number of samples is being input to the step.

The `validateSampleCount` script is triggered when the user clicks the Begin Work button to exit the Ice Bucket screen and start the step.

1. The script compares the number of analyte / derived sample step inputs to the -min and -max parameter values.
2. If the number of inputs:
    - Lies within the -min and -max value range, the user proceeds to the next screen.
    - Is less than the -min value or more than the -max value, user is returned to the Ice Bucket screen.

Count outputs mode

The typical use case for count outputs mode is to ensure that enough replicates (derivatives) are created to fill a flow cell:

1. The `validateSampleCount` script is triggered when the user clicks the Begin Work button to exit the Ice Bucket screen and start the step.
2. The script compares the number of step outputs to the -min and -max parameter values.
    - The script counts all per-input outputs, both analyte / derived sample and result file / measurement types.
    - The script ignores Shared ResultFiles / step file placeholders and pool outputs.
3. If the number of outputs:
    - Lies within the -min and -max value range, the user proceeds to the next screen.
    - Is less than the -min value or more than the -max value, user is returned to the Ice Bucket screen.

Script Parameters

-u {user	(Required) LIMS login username
-p {password}	(Required) LIMS login password
-i {stepURI:v2}	(Required) LIMS step URI
-o <true/false>	(Optional) Set this parameter to true to run the script in count outputs mode. If the parameter is omitted or has a value other than true, the script will run in count inputs mode.
-min <#>	(Optional) Specifies the minimum number of inputs / outputs required. If not provided, default minimum is 0.
-max <#>	(Optional) Specifies the maximum number of inputs / outputs required. If not provided, default maximum is 2147483647.

Command Line Examples

Count inputs mode

Two examples are provided below; both set the script to count inputs mode. In the first example, the -o parameter is provided and set to false. In the second example, the -o parameter is omitted.

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar
```

```
-i {stepURI:v2} -u {username} -p {password} -min 7 -max 8 -o false
script:validateSampleCount"

bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar
-i {stepURI:v2} -u {username} -p {password} -min 7 -max 8
script:validateSampleCount"
```

Count outputs mode

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar
-i {stepURI:v2} -u {username} -p {password} -min 7 -max 8 -o true
script:validateSample
```

Configuration

The `validateSampleCount` script is intended to run when the user clicks the Begin Work button on the Ice Bucket screen and starts the step.

To achieve this, configure the automation trigger as follows:

- Trigger Location: Step
- Trigger Style: Automatic upon entry

Validate Unique Indexes

The Clarity LIMS NGS Extensions Package v5.14 and later includes the `validateUniqueIndexes` script. When defining a batch of samples for library prep, this script ensures that the resulting batch will be valid for later workflow steps - such as pooling or sample sheet generation.

This section provides details on the script, its parameters, and LIMS configuration requirements.

Script Overview

The `validateUniqueIndexes` script checks that the reagent labels (referred to as reagent types in LIMS v4.x and earlier) attached to the input samples have distinct index sequences.

By default, validation will fail:

- If input samples have the same reagent labels.
- If input samples have reagent labels with different names but the same index sequence.
- If the Ice Bucket contains more than one unindexed sample (see Rules & Constraints).

- If any input sample is a pool (see Pool Types). You can change this behavior using the -allowPools command-line option (see Script Parameters).
- If pools are allowed, they will be validated like other input samples.
- Every index sequence applied to step inputs in the pool will be considered when checking for duplicated sequences (see Rules & Constraints).

Pool Types

An input is considered to be a pool if it contains multiple submitted samples and/or multiple reagent labels. A typical pool comprises multiple submitted samples and multiple labels; however, the following less common, and more complex, use cases are also supported.

A submitted pool

- This has one submitted sample, but multiple labels - therefore it is considered a pool.

Multiple submitted samples pooled together without labels

- This has multiple submitted samples - therefore it is considered a pool.

A single submitted sample with replicates recombined back into a pool

- If the recombination is done with adding labels to the replicates, it is considered a pool.
- If the recombination is done without adding labels, it is not considered a pool.

Script Parameters

-u {username} -username {username}	(Required) LIMS login username
-p {password} -password {password}	(Required) LIMS login password
-i {stepURI:v2} -stepURI {stepURI:v2}	(Required) LIMS step URI
-allowPools <true/false>	(Optional) Default is false. When provided and set to true, the script will allow pools as inputs to the step. By default, validation will fail if any input contains multiple submitted samples or multiple reagent labels.

Command Line Example

Example 1

```
bash -c "/opt/gls/clarity/bin/java -jar \
```

```
/opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar
-i {stepURI:v2:http} \
-u {username} \
-p {password} \
script:validateUniqueIndexes"
Note that this command line also uses the Sample Placement Helper script
(PlacementHelper.jar), included in the Lab Instrument Toolkit.
```

Example 2

```
bash -c "/opt/gls/clarity/bin/java -jar \
/opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar \
-i {stepURI:v2:http}
-u {username}
-p {password}
script:validateUniqueIndexes
-allowPools true"
```

Configuration

The `validateUniqueIndexes` script is intended to run when the user selects the Begin Work button on the Ice Bucket screen and starts the step.

To achieve this, configure the automation trigger as follows:

- Trigger Location: Step
- Trigger Style: Automatic upon entry / exit

Rules and Constraints

- Each reagent label name must be unique. The label name is only used to look up the corresponding index sequence in the API. However, if two or more label groups (referred to as reagent categories in LIMS v4.x and earlier) contain labels with the same name, the LIMS will not be able to distinguish between them and the validation script will fail.
- The script does not look upstream for labels - it only looks at the labels currently applied to the step inputs.
- The Ice Bucket may include one input sample with no index assigned, therefore allowing a PhiX or similar control to be included in the pool (a control will typically have no label - this scenario is supported and the validation script will run without error).
  For LIMS v4.x and earlier, this also applies to samples with a reagent type that is not an index. Note, however, that in any scenario, the label must exist as a reagent type in the LIMS. If you add a label to an input, and the label does not have a corresponding reagent type, the validation script will fail. See note below.

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In LIMS v4.x and earlier, a reagent type may not be an index type. In this case, it will have a label and a corresponding reagent type, but no sequence value. This scenario is supported and the reagent type is treated as non-indexed. It is also possible, via the API, to add labels arbitrarily to samples, without those labels having a corresponding reagent type - this scenario is not supported.

Configure Multiple Identical netPathPrefixSearch Values

It is possible to provide support for multiple, identical seqservice.netPathPrefixSearch property values. This is achieved by using the seqservice.eventFileDirsToUseForSearchReplacePair properties. These properties can be used to specify the mappings between search replace pairs and their associated event directories.

- The value of the seqservice.eventFileDirsToUseForSearchReplacePair property is a comma-separated list of event file directory suffixes to be applied to the search/replace pair.
- If the property is not supplied (or the value is left empty), the behavior is unchanged. In this case, the search/replace pair is treated as global and applied to all active event file directory suffixes.

By default, BaseSpace Clarity LIMS does not specify the eventFileDirsToUseForSearchReplacePair property. If you want the search/replace pair to apply only to a subset of the event file directories, you must supply the property manually.

The following example uses a HiSeq installation to show several configuration options, with comments. Similar configuration may be applied for installations of other Illumina instrument integrations (eg, MiSeq, NextSeq).

Note that the last part of this example shows configuration that results in an error.

```
hiseqga.seqservice.eventFileDirectory.1 '/mnt/Hiseq1/data/gls_events'


hiseqga.seqservice.eventFileDirectory.2 '/mnt/Hiseq2/data/gls_events'


hiseqga.seqservice.eventFileDirectorySuffixes '1,2'




hiseqga.seqservice.netPathPrefixSearch.1 'Y:\'
```


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```
hiseqga.seqservice.netPathPrefixReplace.1 '/mnt/Hiseq1/data/'

hiseqga.seqservice.eventFileDirsToUseForSearchReplacePair.1 '1' // just use search/replace
pair 1 in dir 1



hiseqga.seqservice.netPathPrefixSearch.2 'Y:\'

hiseqga.seqservice.netPathPrefixReplace.2 '/mnt/Hiseq2/data/'

hiseqga.seqservice.eventFileDirsToUseForSearchReplacePair.2 '2' // just use search/replace
pair 2 in dir 2



hiseqga.seqservice.netPathPrefixSearch.3 'A:\'

hiseqga.seqservice.netPathPrefixReplace.3 '/mnt/Hiseq2/data/'

hiseqga.seqservice.eventFileDirsToUseForSearchReplacePair.3 '1,2' // use search/replace pair
3 in dir 1 AND 2



hiseqga.seqservice.netPathPrefixSearch.4 'B:\'

hiseqga.seqservice.netPathPrefixReplace.4 '/mnt/Hiseq2/data/'

hiseqga.seqservice.eventFileDirsToUseForSearchReplacePair.4 '' // global, use this
search/replace pair in all dirs



hiseqga.seqservice.netPathPrefixSearch.5 'C:\'

hiseqga.seqservice.netPathPrefixReplace.5 '/mnt/Hiseq2/data/' // no replace pair dir prop
specified = global, use this search/replace pair in all dirs



hiseqga.seqservice.netPathPrefixSearch.6 'Y:\'

hiseqga.seqservice.netPathPrefixReplace.6 '/mnt/Hiseq2/data/'

hiseqga.seqservice.eventFileDirsToUseForSearchReplacePair.6 '1' // duplicate search value
(Y:) for event file dir 1 - ERROR



hiseqga.seqservice.netPathPrefixSearchReplaceSuffixes '1,2,3,4,5,6'
```


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Illumina Instrument Integrations FAQ

The Clarity LIMS integration with Illumina instrument software provides the following features:

- Instrument monitoring
- Automated reporting
- Capturing and parsing run statistics

This section answers questions that are often asked about Illumina integration packages.

To ensure that your Illumina instrument warranty remains valid, the instrument integration must be performed and maintained by the Clarity LIMS Support team.

Will installing this software impact our Illumina instrument warranty?

The Clarity LIMS instrument integrations are designed to communicate with Illumina instruments. To ensure that your Illumina instrument warranty remains valid, the instrument integration must be performed and maintained by the Clarity LIMS Support team. Under this agreement, the LIMS software is guaranteed to not void or otherwise impact your instrument warranty in any way.

What Illumina software is required for the integration?

Clarity LIMS requires a minimum version of the controller software, and access to sequencer instrument workstations for the installation of a batch file script that is triggered at select sequencing run events (start run, cycle complete, and run complete). Requirements are as follows:

- GAII, GAIIx running SCS 2.9 (RTA 1.9), SCS 2.10 (RTA 1.13)
- HiSeq 2000/1000 running HCS 1.4 (RTA 1.12), HCS 1.5 (RTA 1.13)
- HiSeq 2500/1500 running HCS 2.0.3 (RTA 1.17)
- CASAVA 1.8.0, 1.8.1, or 1.8.2 for Bcl conversion / demultiplexing
- HiSeq X running HCSX 3.1 (RTA 2.3)
- MiSeq running MCS 1.1 (RTA 1.13), MCS 2.0 (RTA 1.16), MCS 2.2 (RTA 1.17)
- NextSeq running NCS 1.2 (RTA 2.1)

What are the individual components of the integration package and how do they interact with the instrument and Illumina software?

Illumina provides a supported mechanism for invoking custom scripts on key events during a sequencing run, and batch files that plug into these events.

The LIMS local script takes the context information passed to it from the Illuminasupported event infrastructure and writes to a text file in a dedicated folder (not the run directory). This folder is located on the same run data network share and is consumed by the Illumina NGS Packages event-processing software running on the LIMS server.

This specific methodology protects both the Illumina instrument and Clarity LIMS by:

- Using only the supported external script integration points supplied by Illumina.
- Using a simple batch file invoked by an Illumina supported mechanism.
- Not conducting any read or write of any data on the instrument itself.
- Not conducting any write of any data to the instrument run directory.
- Not requiring any software to be installed on the instrument machines (no installer to run, no changes to system registry, no changes to environment variables, etc.).
- Not requiring an install of Automation Worker (AW) node on the Illumina computer.

Why use event files, and require the mounting of the NAS file system?

The central point of network contact and connectivity between Clarity LIMS and the Illuminainstrumentation is the Network Attached Storage (NAS).

The NAS is used as the hub and interprocess communication (IPC) mechanism to ensure that:

- No additional network paths, ports, or connections need to be configured on-site. The Clarity LIMS server only has the same access to the NAS that the Illumina instrument (Windows®) and Bcl conversion server (Linux) already has. By not requiring direct network connectivity between the Illumina instruments and the LIMS server, the amount of work required by your IT department to enable this integration is minimized.
- No additional file access protocols need to be configured. The Windows-based Illumina instrument writes to the NAS using a standard file read. The Linux-based Bcl conversion server requires similar file-share-based access to operate.
- Robustness of the NAS is implied because:
  - For the Illumina Instrument to run, it must be able to read/write its data to this NAS location.
  - For the Bcl conversion to run, it must already be able to read/write its data to this NAS location.

How does the Clarity LIMS server access information on the NAS?

The NAS is mounted using a standard Linux mount using NFS or CIFS.

How are the event files managed. Are there any issues expected with files building up over time?

The general paradigm is one of consumption. This means that when an event file produced by the Clarity LIMS NGS integration has been properly processed, it is moved to an archive directory sorted by year and month.

If a Begin Run or End Run event file is found, and there is no corresponding flow cell/reagent cartridge in the system (or there is insufficient data in the run folder at this time to process it), that event file is left for processing the next time the daemon looks for events.

If a Begin Run or End Run event file is not processed within 14 days, the event is archived to the directories sorted by year and month. When an event is archived, the event file is read and the container name is pre-pended to the event file name, making it easier to find all events for a container.

If a Cycle Complete event file is found and there is no corresponding flow cell in the system, that event is consumed and removed.

How does Clarity LIMS deal with runs externally removed from the file system/NAS?

The Clarity LIMS Illumina NGS integration package itself does not support this scenario. The LIMS interface operates without problems while the instrument is running (capturing run parameters, updating status, capturing and parsing statistics, and generating reports). However, after sequencing run completion, if you move the data to another location, the process fields and the generated link files will no longer be valid.

If you want to subsequently run the Bcl Conversion and Demultiplexing step, it will attempt to locate the network run data (via the custom field configured on the sequencing process), and will fail since the data is no longer there.

The minimum manual update required is to change the Output Folder field on the Illumina Sequencing (Illumina SBS) step— from the original location of the run data directory to the location of the data that will be used for the Bcl Conversion and Demultiplexing (Illumina SBS) step run. Finally, updated he Output Folder field value to the location of the permanent home of the run data on the network storage.

If you move the data after both Sequencing and Bcl Conversion and Demultiplexing steps are complete, the links to the run data and demultiplexed sample directories and calculated metrics will no longer be valid.

What happens to event files if a run is started with a sample sheet not created by Clarity LIMS (thus with an unknown flow cell)?

If the flow cell is unknown, the Begin Run and End Run events build up in the gls_events folder and the Cycle Complete events are removed. As there is no corresponding Flow Cell ID in the LIMS, no information regarding the run is recorded.

How are FASTQ files delivered (Bcl Conversion and Demultiplexing) in Clarity LIMS?

When the Bcl Conversion and Demultiplexing step completes, it attaches HTML link files to the FASTQ result file output placeholders in the LIMS. These files contain links to the demultiplexed sample directories and calculate metrics. The `Demultiplex_Stats.htm` and log file are attached, and the LIMS parses `Flowcell_demux_summary.xml`.

Do we know (from file system level) when Clarity LIMS is finished reading from and writing to a specific run?

There is no direct indication at the file system level. Run complete events created in gls_events are consumed and the link to the run directory is created in Clarity LIMS.

Other files are created (reports, for example) and are attached directly to the LIMS. These files do not appear in the file system, so there is no clear indication when the LIMS has finished from this level.

However, a user can tell when a sequencing run is complete, or at what stage it is at, through the Clarity LIMS interface:

- The Sequencing step displays a Finish Date and Cycle Status.
- The system also generates a link back to the run folder where the data was captured.
- The `RunReport.pdf` is generated. This is the last step of the Sequencing process. Once this is associated in the LIMS, the step is complete.

While it is not possible to determine that the LIMS has finished processing at the file system level directly, it is possible to determine that processing has finished by querying the REST API. This requires custom scripting by the customer, but may provide a mechanism to detect when to initiate the next stage of the analysis pipeline, in the scenario that the Bcl Conversion and Demultiplexing integration with CASAVA does not meet requirements.

Illumina instrument users can select multiple NAS drives in the output folder prompt of the Illumina control software. How does Clarity LIMS handle this?

If you require configuration of multiple NAS drives, you will need to provide the Clarity LIMS Support team with the details of all of your drive locations.

What are the permissions required for Illumina instrument run data directory?

The Illumina NGS Package sequencer instrument interface running on the LIMS server was designed to require a Read/Write mount to the network run data folder root for the instruments. This allows for maximum automation and convenience.

In addition, it allows for automation of subsequent Bcl conversion and demultiplexing with a supplied `SampleSheet.csv` file. The file is required by the CASAVA/Bcl Conversion and Demultiplexing step.

Note the following:

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- For HiSeq/GAIIx and HiSeq X, the `SampleSheet.csv` file is generated by and attached to the Cluster Generation step in Clarity LIMS
- For MiSeq and NextSeq, the `SampleSheet.csv` file is generated by and attached to the Denature, Dilute and Load Samples step.
- The file may be used with CASAVA for HiSeq/GAIIx, so is placed in the `\<Run>\Data\Intensities\BaseCalls\` directory. This is where CASAVA/Bcl Conversion and Demultiplexing looks for the file.

NextSeq and HiSeq X deliver a file for use with Illumina's bcl2fastq v2 tool.

Alternative option

If necessary, you can mount the run data directory with 'Read Only' access.

The following actions still occur and function correctly:

- The sequencing master step fields are populated with key run information.
- The per-lane statistic custom fields on the flow cell are populated with run summary statistics.
- The `SampleSheet.csv` file iscreated and attached in the LIMS.
- The `runreport.pdf` is created and attached in the LIMS.

The following actions no longer occur and fail:

- The `SampleSheet.csv` file is not be written to <runFolderRoot>/Data/Intensities/BaseCalls/
- A log file message records that the `SampleSheet.csv` failed to be written.

> If this alternative option is used, the instrument must be able to write to the network location, and the Clarity LIMS server must have Read/Write/Delete permissions to the network location. This folder location is defined in the `hiseqga.seqservice.eventFileDirectory` database property, and is used for the monitoring and consumption of instrument-generated event files.

How does the Clarity LIMS NGS Package handle network issues between the Illumina Instrument and NAS share?

Illumina instruments write locally, with a worker process run-time access (RTA) daemon. This service is responsible for moving data from the local drive to the network drive that is the actual target directory.

Both Clarity LIMS and the Illumina instrument generally assume that the network drive is a reliable resource. The Illumina software is only so tolerant of outages and much depends upon the duration (seconds, minutes, hours, days, weeks, etc.). Clarity LIMS has a general assumption that the network location will be reliable.

If there is an outage, the following may occurs:

- If a network resource is unavailable, there is no adverse effect upon the instrument processing. Specifically, if the batch file attempts to write the event file, and the network drive cannot be accessed, it does not block or pause normal instrument operation.
- If a network resource is unavailable when the batch file attempts to write the event text file. That event is abandoned and is never successfully written if the first attempt fails.

In practice, these scenarios are rare and seldom cause problems.

- If the events do fail to be written, the Begin Run and End Run events can be created manually. The run is still recorded accurately in Clarity LIMS. Note that there is no specific real-time dependency on when that run is registered in the LIMS.

How does Clarity LIMS interact with the Illumina RTA Software?

Illumina RTA software operates by first writing to a local disk. RTA2 software instead operates in memory and then uses an HTTP interface to transfer information to the HCSX software. During the run, it analyzes the run data and transfers it to the Illumina network run directory, where it is available for access once the run completes. Clarity LIMS does not interact with or monitor the local drive directly; it only monitors the network run directory to which RTA streams the data during the run.

Does Clarity LIMS capture confidential data of runs for customers that do not have their work recorded in the LIMS?

Clarity LIMS only captures and records data if there is a match between Flow Cell IDs in the LIMS and on the instrument run. Flow Cell IDs are recorded differently, depending on the instrument type.

HiSeq and HiSeq X—Recorded in the runParameters.xml `<Barcode />` property

MiSeq—Recorded in the runParameters.xml `<ReagentKitRFIDTag><SerialNumber />` property

NextSeq—Recorded in RunParameters.xml `<ReagentKitSerial>` property

If there is no match, no data are captured or recorded. If there is a match, the following types of data are recorded:

- Statistical data associated to Cluster Generation (Yield PF, Avg Q Score, % Bases etc.)
- Status data associated to Sequencing (Cycle Status, Flow Cell ID, Flow Cell Position, Read 1/2 Cycles, Finished Date etc.)
- Generated reports (Run Report, which includes Clarity LIMS sample/project names, run and read cycle summaries etc.)
- Clarity LIMS does not capture the raw data. Instead, it provides HTML links to the locations in which this data is stored on the NAS Share—specifically, to the following:
  - Run Data directory
  - Demultiplexed sample directories and calculated metrics

Avg Q Score is not present for HiSeq X and is being phased out of other instrument integrations.

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Do any Bcl Server issues result from cluster & grid computing environments?

To automate the dispatch and invocation of Bcl conversion and demultiplexing jobs, Clarity LIMS NGS Packages require our lightweight EPP/automation program to be installed and run on the CASAVA (Bcl) server. Some customers are uncomfortable with this, do not desire this automation, or are moving the data pre and post Bcl conversion and demultiplexing.

Normal Operation

The automation/EPP program receives dispatches from the LIMS server and runs a locally-deployed script (embedded inside the ngs-extensions.jar) to do the following:

- Access the LIMS server API to gather the information required to generate command lines for the `configure` and `make` commands.
- Invoke the `configure` and `make` commands to perform Bcl conversion and demultiplexing.
- Wait for Bcl conversion and demultiplexing completion.
- Parse `Flowcell_demux_summary.xml` to get sample names and statistics to store in the LIMS.
- Traverse the `Unaligned` directory for Project/Sample folder names to create the HTML link files.

Alternative Options

Customers who are unable or unwilling to install EPP on their CASAVA (Bcl) server will not be able to automate CASAVA (Bcl) runs. However, they can still benefit from two elements of the integration:

- Generation of command lines for CASAVA (Bcl).
- Storage of the post-demultiplexing sample statistics and file locations in Clarity LIMS.

Configuration Required

Modify the configuration of the Bcl Conversion and Demultiplexing (Illumina SBS) Clarity LIMS step as follows.

1. On the automation, update the channel to limsserver.
2. Update the EPP/automation command line string in such a way that:
   - It would work properly if it was installed on the CASAVA (Bcl) server.
   - The java path (`/usr/bin/java`) references the local LIMS server.
   - The path to the ngs-extensions.jar file (`/opt/gls/hiseqgaii-extensions.jar`) references the local LIMS server (`/opt/gls/clarity/extensions/Illumina_HiSeq/v5/EPP/hiseqgaii-extensions.jar`).
   - -m {udf:MODE} is appended to the command line.
3. Add a master step field/process UDF called MODE and assign it two values:
   - SIMULATE

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- HARVEST

4. Ensure that you have completed the standard configuration for the following properties:
   - `hiseqga.bcl.netPathPrefixSearch.1`
   - `hiseqga.bcl.netPathPrefixReplace.1`
   - `hiseqga.bcl.replacementSuffixes.limsserver`

   The `hiseqga.bcl.replacementSuffixes.limsserver` property is not created by default. Add this property manually. You must do one of the following:
   - Ensure that the mount name and path through which the LIMS server 'sees' the network run data directories is the same as that through which the CASAVA(Bcl) server 'sees' these directories.
   - Ensure that additional `netPathPrefixSearch` and `netPathPrefixReplace` properties are configured and that the `hiseqga.bcl.replacementSuffixes.limsserver` maps to those numeric indices.

AThe `bcl.replacementSuffixes` property formerly shared a set of search/replace paths with the sequencing `netPathPrefixSearchReplaceSuffixes`. This is no longer the case; it now references its own `bcl.netPathPrefixSearch` and `bcl.netPathPrefixReplace` properties.

Use the Updated Configuration

SIMULATE Mode

- Optional) To run CASAVA (Bcl), run the Bcl Conversion and Demultiplexing (Illumina SBS) step and select step and select SIMULATE. This generates all the standard command lines, which are recored in the `Script Log Details` log file attached to the step. You can then use these command lines to set up the run manually on the CASAVA (Bcl) server at the command line. In SIMULATE mode, the script does everything it would have done in terms of generating command lines, but does not actually invoke anything.

HARVEST mode

- (Optional) When the CASAVA (Bcl) run completes, to capture statistics, run the Bcl Conversion and Demultiplexing (Illumina SBS) step and select HARVEST. In HARVEST mode, the script looks at the run folder, finds the `Flowcell_demux_summary.xml` file, parses the relevant data and stores everything it normally would in the LIMS. HARVEST mode also traverses the Unaligned directory for the necessary information to create the HTML link files.

How much space do event files take up?

Event files take up very little space on disk. See the following calculation example for 6 HiSeqs running at full capacity. Note that MiSeqs and NextSeqs will use up even less space for their event files. It is up to the customer to determine how much space is needed for the actual sequencing data, this discussion

is only about the incremental space required for LIMS event files.

- Each HiSeq Run creates two event files which are archived: 1x Begin Run and 1 x End Run event file. The End Imaging (or cycle complete) event files are consumed in real-time and not archived.
- Each event file is between 180 and 220 bytes in size.

Suppose each HiSeq runs in Rapid mode so completes a run every single day. Assume the lab has 6 HiSeqs.

- 6 runs completed each day X 365 days = 2190 runs per year
- 2190 runs per year X 2 event files/run = 4380 event files produced annually
- 4380 event files/year X 220 bytes = 963600 bytes, which converts to 0.91 MB (1,048,576 Bytes in a megabyte)

Revision History

Document	Date	Description of Change
Document # 1000000150228 v11	June 2024	Added the following packages to support IPP v2.8: • NextSeq 1000/2000 On-Premise Integration v1.0 • NextSeq 1000/2000 Integration v2.5 • NGS v5.25 Updated CLPA v1.3 integration information.
Document # 1000000150228 v10	February 2024	Added the following packages to support IPP v2.7: • NovaSeq X Series Integration v1.2 • NGS v5.24 Added information about how inputting pools with only one sample prints out the pooled artifact to *Template File Contents*. Added demultiplexing conditions to the NextSeq 1000/200 documentation (v2.0–v2.4). Updated compatibility table for Clarity LIMS Product Analytics v1.2. Updated release notes for NextSeq 1000/2000 (v2.0–v2.4).

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Document	Date	Description of Change
Document # 1000000150228 v09	September 2023	Added documentation for the following integration packages to support IPP v2.6: • Clarity LIMS Product Analytics Integration v1.2 • iScan Integration v1.1 • MiSeq Integration v8.2 • MiSeqDx Integration v1.10 • NextSeq 1000/2000 Integration v2.4 • NextSeq 500/550 Integration v2.3 • NGS v5.22 • NGS v5.23 • NovaSeq 6000 Integration v2.5 • NovaSeq 6000 Integration v3.6 • NovaSeq 6000Dx Integration v1.2 • NovaSeq X Series Integration v1.1
Document # 1000000150228 v08	July 2023	Added documentation for the following integration packages to support IPP v2.5: • NovaSeq X Series Integration v1.0 • NovaSeq 6000Dx Integration v1.1 • NovaSeq 6000 Integration v3.5.1 • NovaSeq 6000 Integration v3.5.0 • NovaSeq 6000 Integration v2.4.1 • NextSeq 1000/2000 Integration v2.3.1 • Clarity LIMS Product Analytics Integration v1.1
Document # 1000000150228 v07	April 2023	Added documentation for the following integration packages to support IPP v2.4: • NovaSeqDx Integration v1.0 • iScan Integration v1.0 • NextSeq 1000/2000 Integration v2.3 • Clarity LIMS Product Analytics Integration v1.0 Under Packages and Reference, added known issues related to the sample placement helper script to the NGS Extensions v5.21.0 Release Notes.

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Document	Date	Description of Change
Document # 1000000150228 v06	March 2022	Added documentation for the following integration packages to support the release of Clarity LIMS v6.0: • NovaSeq 6000 Integration v3.4 • NovaSeq 6000 Integration v2.4 • NextSeq 1000/2000 Integration v2.2 • NextSeq 500/550 Integration v2.2 • MiSeq Integration v8.1 • MiSeqDx Integration v1.9 Under Packages and Reference, added NGS Extensions v5.21.0 Release Notes.
Document # 1000000150228 v05	January 2022	Added NextSeq 1000/2000 Integration v2.1.1 Release Notes. Under Packages and Reference, moved NGS documentation into separate subsection.
Document # 1000000150228 v04	August 2021	Added NextSeq 1000/2000 Integration v2.1. Added NGS Common Package v5.20.3 Release Notes.
Document # 1000000150228 v03	June 2021	Added MiSeq Integration v8.0.
Document # 1000000150228 v02	April 2021	Added NextSeq 1000/2000 Integration v2.0.
Document # 1000000150228 v01	February 2021	Added MiSeq Integrations v7.0 and v7.1.
Document # 1000000150228 v00	January 2021	Initial release in new help format.

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